TCA Cycle Pathway

Dr. Manal Bayouni
Professor of Biochemistry & Molecular Biology

TCA cycle is also called

The Krebs Cycle

or

The Citric Acid Cycle

It plays several roles in the metabolism

Krebs Cycle

Is a series of reaction that occurs in aerobic cells Order to make complete oxidation of AcetylCoA derived from carbohydrates, fats, and proteins into CO_2 & chemical energy in the form of (ATP)

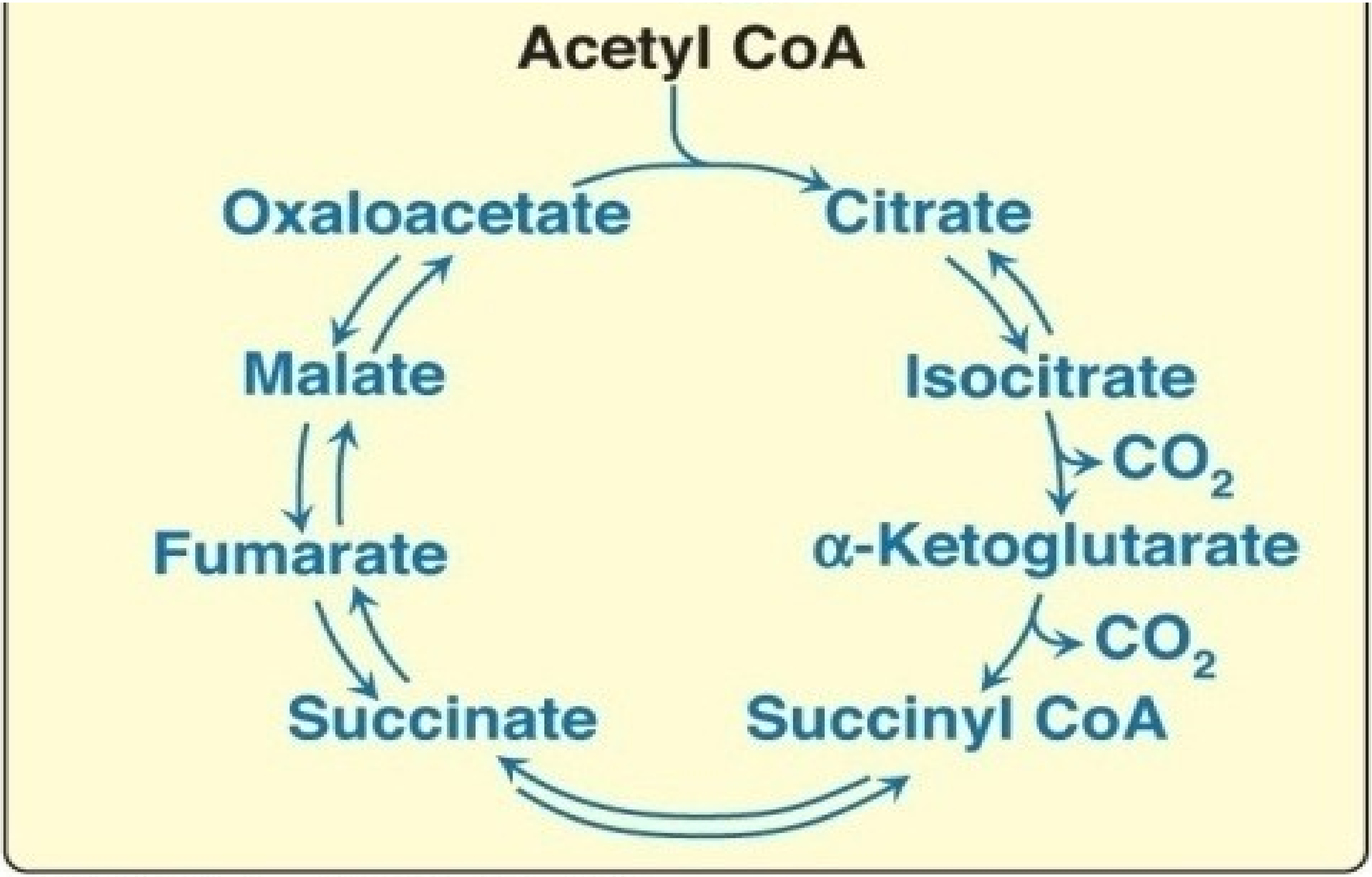
TCA cycle occurs only in mitochondrial matrix

By another meaning takes place only in aerobic cell

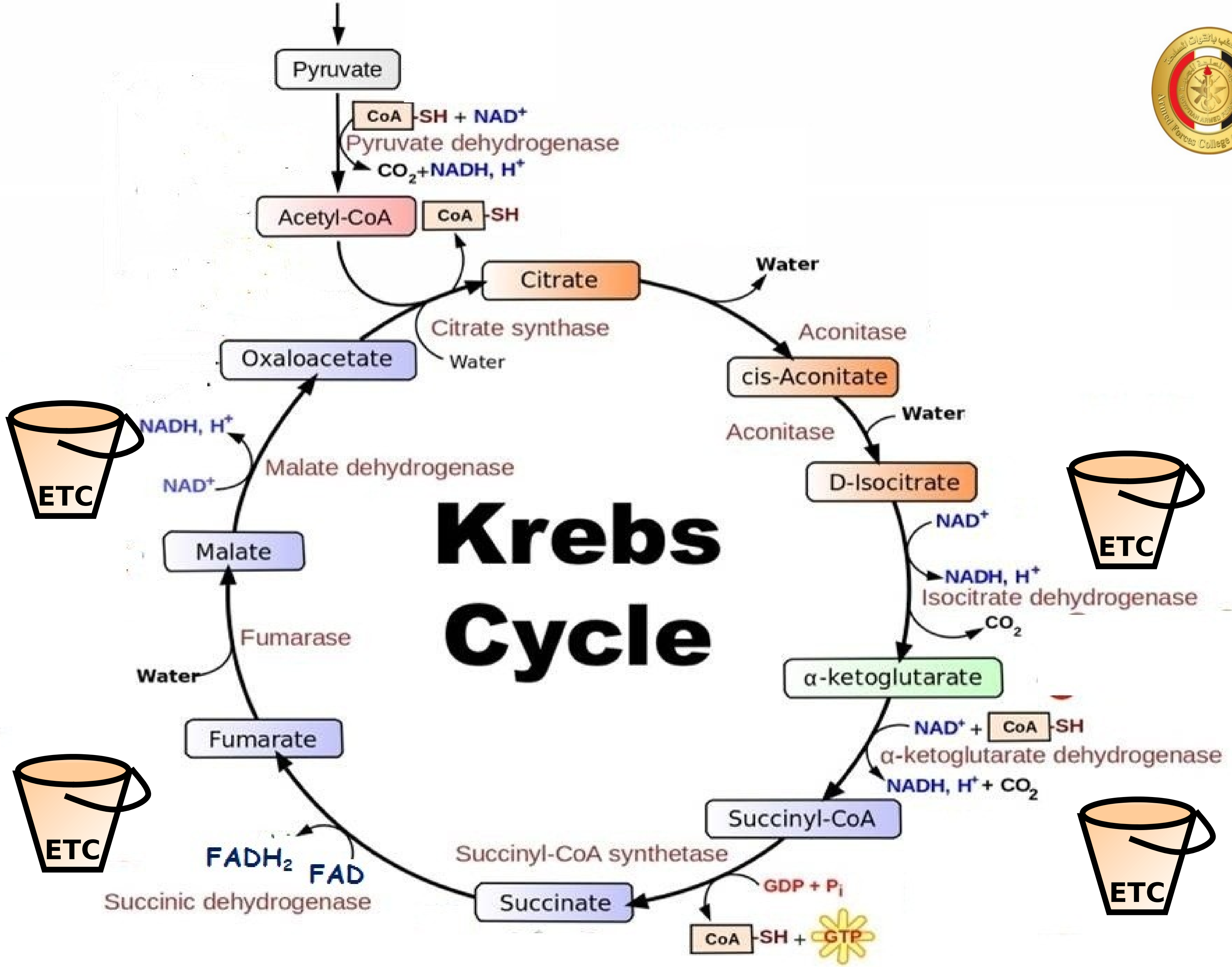
Because its products like $NADH+H^+$ & $FADH_2$

Must be reoxidized back to NAD & FAD BY electron transport chain which consume O_2 and produce ATP & H2O

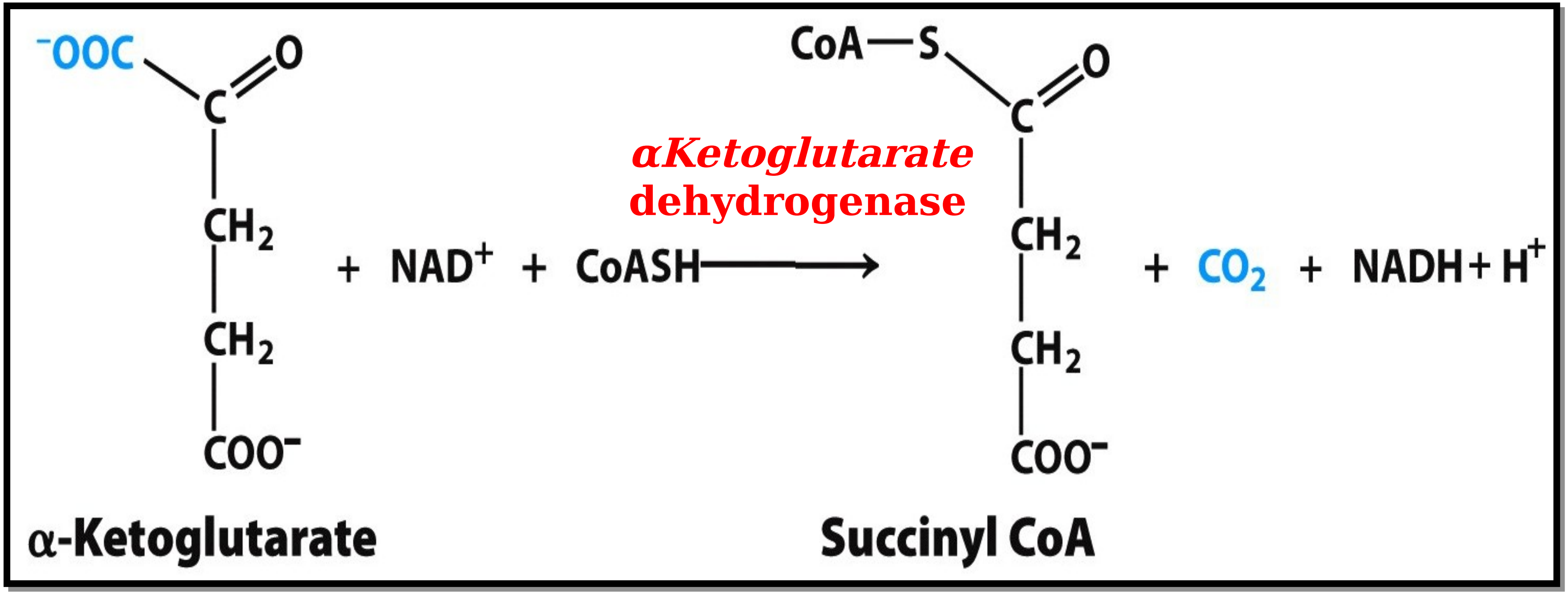
Krebs Cycle



Acetyl CoA
Oxaloacetate
Citrate
Malate
Isocitrate
CO_2
Fumarate
α-Ketoglutarate
CO_2
Succinate
Succinyl CoA

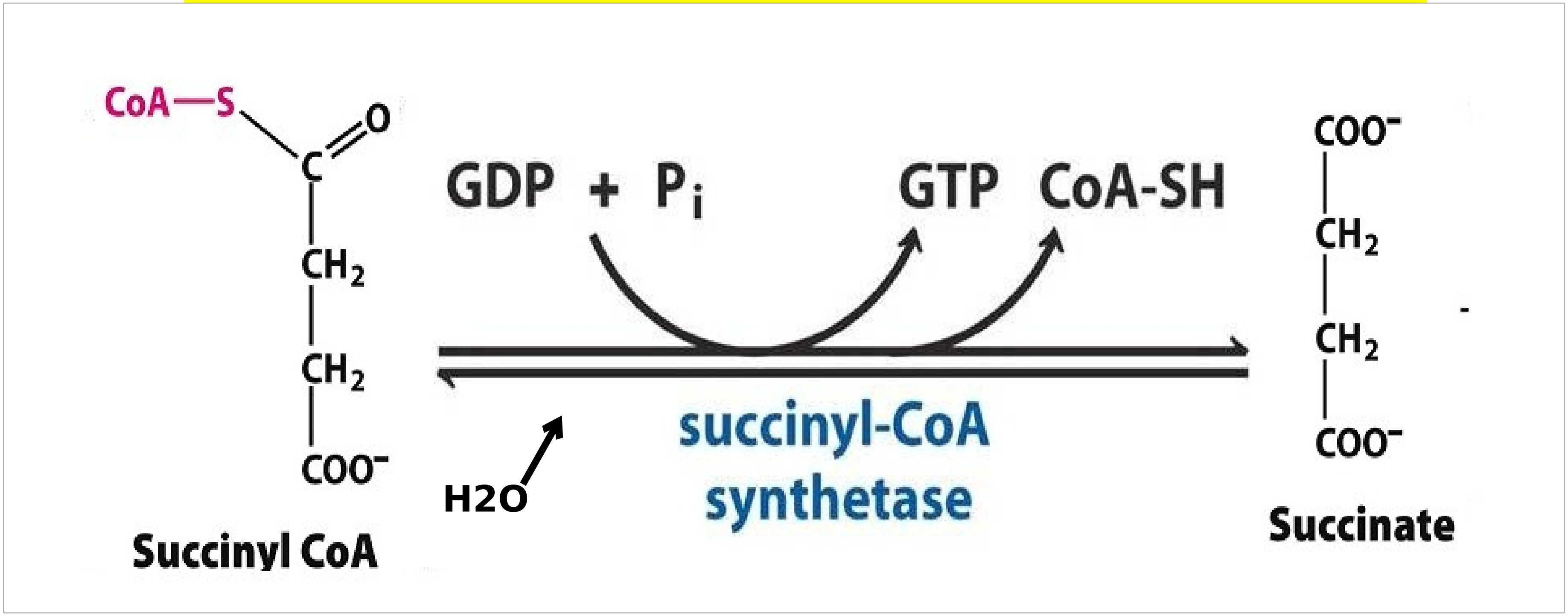

Pyruvate
CoA -SH + NAD+
Pyruvate dehydrogenase
CO2+NADH, H+
Acetyl-CoA
CoA -SH
Citrate
Citrate synthase
Water
Water
Aconitase
cis-Aconitate
Water
Aconitase
D-Isocitrate
NAD+
NADH, H+
Isocitrate dehydrogenase
CO2
α-ketoglutarate
NAD+ + CoA -SH
α-ketoglutarate dehydrogenase
NADH, H+ + CO2
Succinyl-CoA
Succinyl-CoA synthetase
GDP + Pi
CoA -SH + GTP
Succinate
Succinic dehydrogenase
FADH2
FAD
Fumarate
Fumarase
Water
Malate
Malate dehydrogenase
NAD+
NADH, H+
Oxaloacetate
Krebs Cycle
ETC
ETC
ETC
ETC

α Ketoglutarate Dehydrogenase



It is enzyme for Oxidative decarboxylation (Typical as PDH) BUT Does Not Subjected to Covalent Modification Like PDH.

Succinate Thiokinase = Succinyl-CoA Synthetase



In this reaction the energy rich thioester linkage is hydrolyzed with release of energy that is conserved in
substrate level of phosphorylation of GDP to GTP

Energy Yield Of The TCA??

TCA = 3 (NADH)+ 1 (FADH2)+ 1 ATP

= 3 x 3 + 1x 2 + 1

= 12 ATP

To summarize

in TCA the 2 carbons of acetyl CoA

are

oxidized and released in the form

of CO_2, with concomitant

formation of

How many molecules of ATP are produced by complete oxidation of one glucose molecule aerobically??

- **Glycolysis= 8**
- **2 PDH= 6 ATP**
- **TCA = 3 (NADH)+ 1 ($FADH_2$)+ 1 ATP**

3x3 + 1x 2 =
+ 1

1 + 2 + 9 =

ATP/ 1 AcetylCoA 12=

Acetyl CoA= 12 X 2= 24 2

= 8+6+24

38 ATP

Biological Importance of TCA = **Amphibolic** Nature of TCA (Its relation to other pathways)

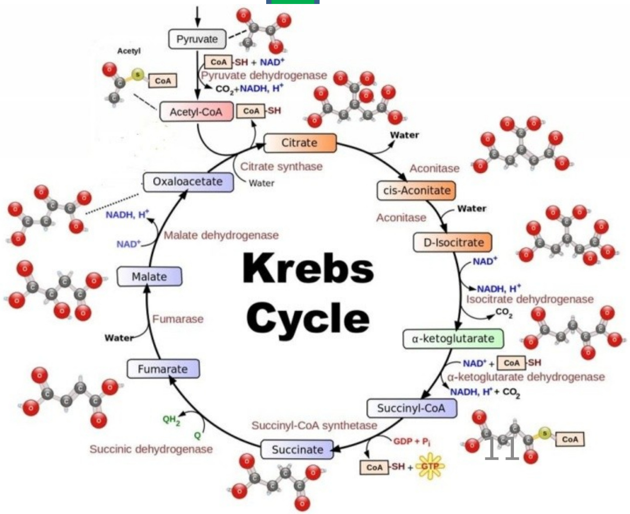
❑Catabolic (oxidative) Nature Of TCA Cycle "Convergent"

Glucose → **Pyruvate** → **Acetyl COA**

Fatty acids oxidation → **Acetyl COA**

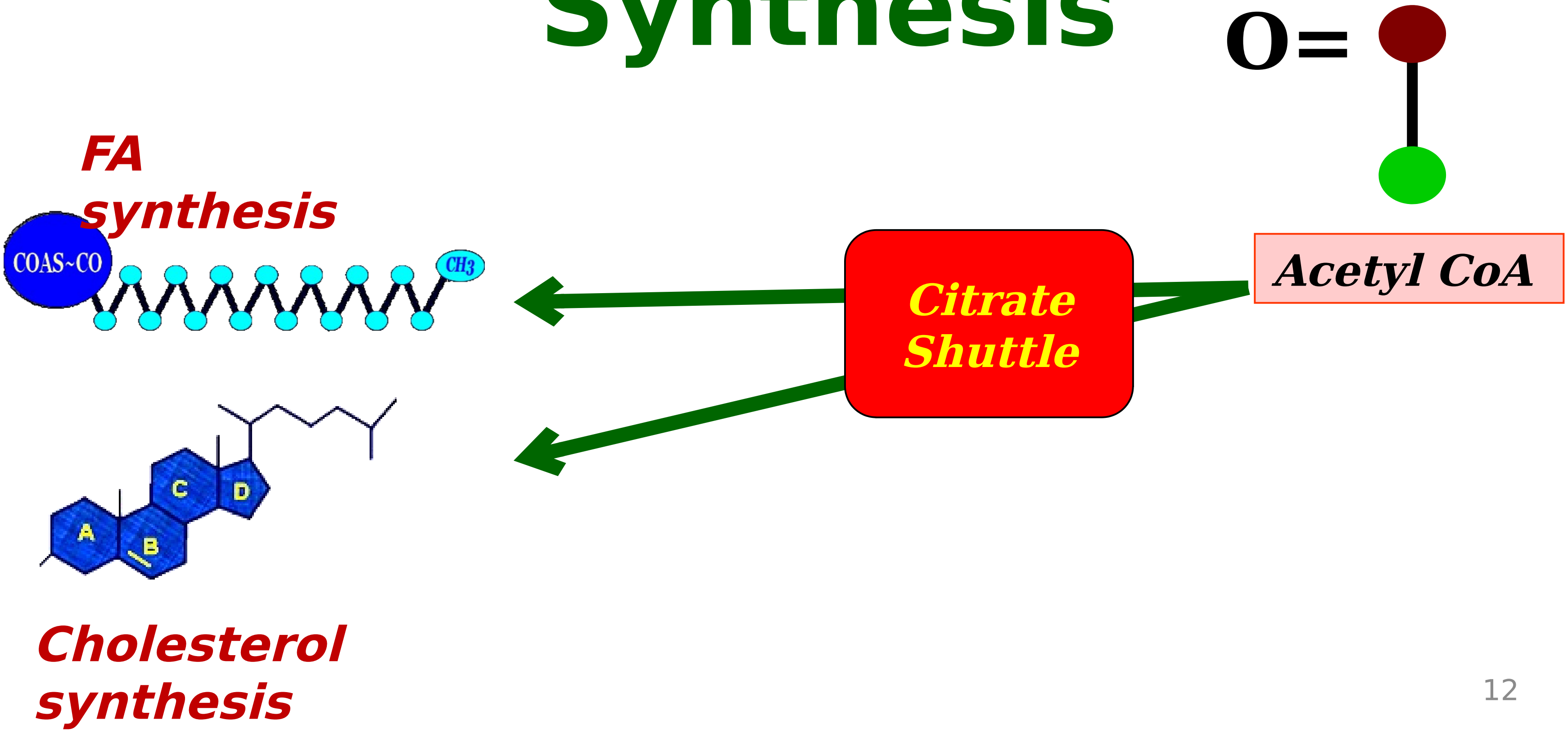
Ketone bodies by ketolysis → **Acetyl COA**

Some aminoacids catabolism (ketogenic) → **Acetyl COA**



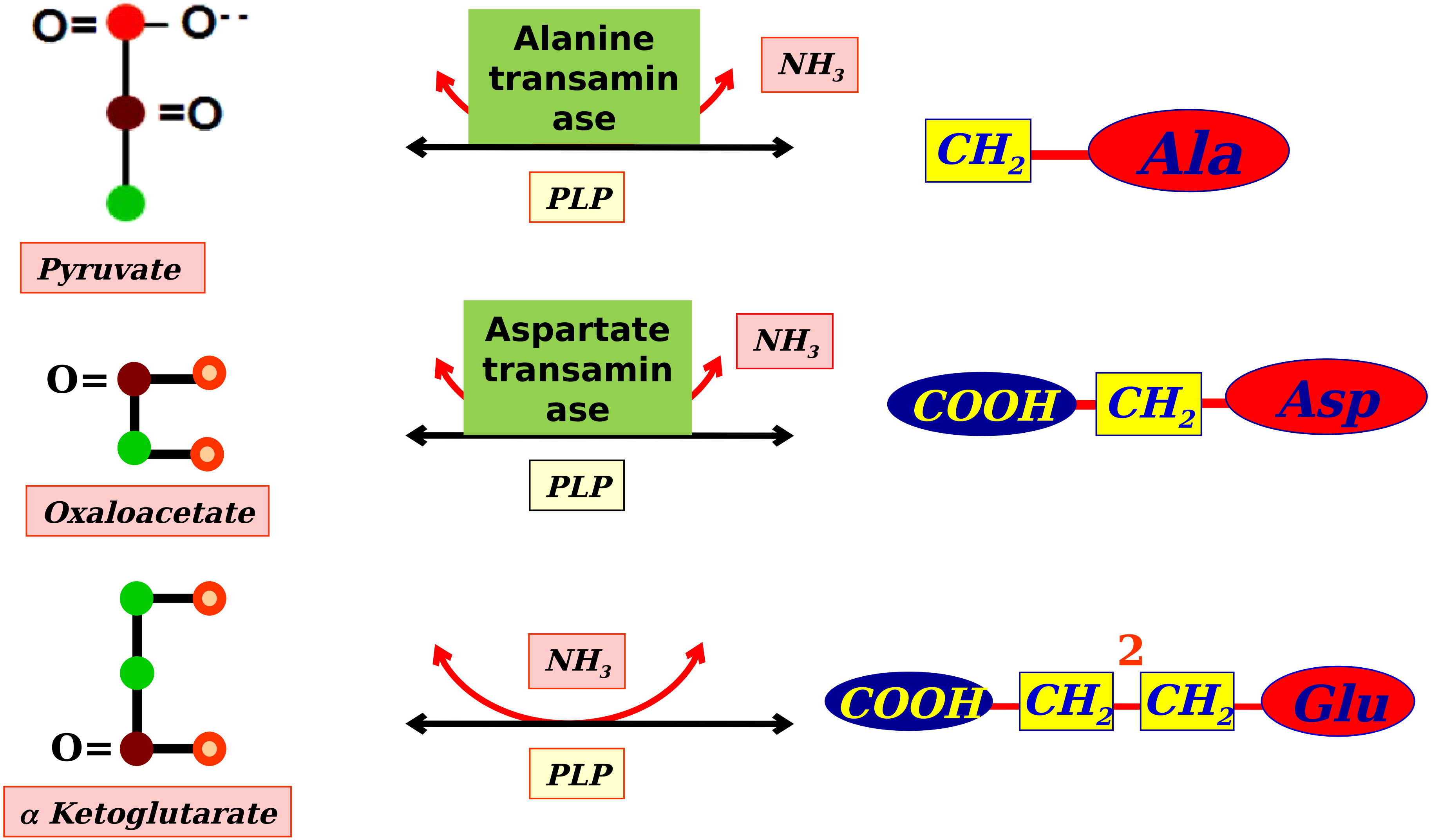
Anabolic Nature Of The TCA Cycle (Divergent)

1. FA & Cholesterol Synthesis

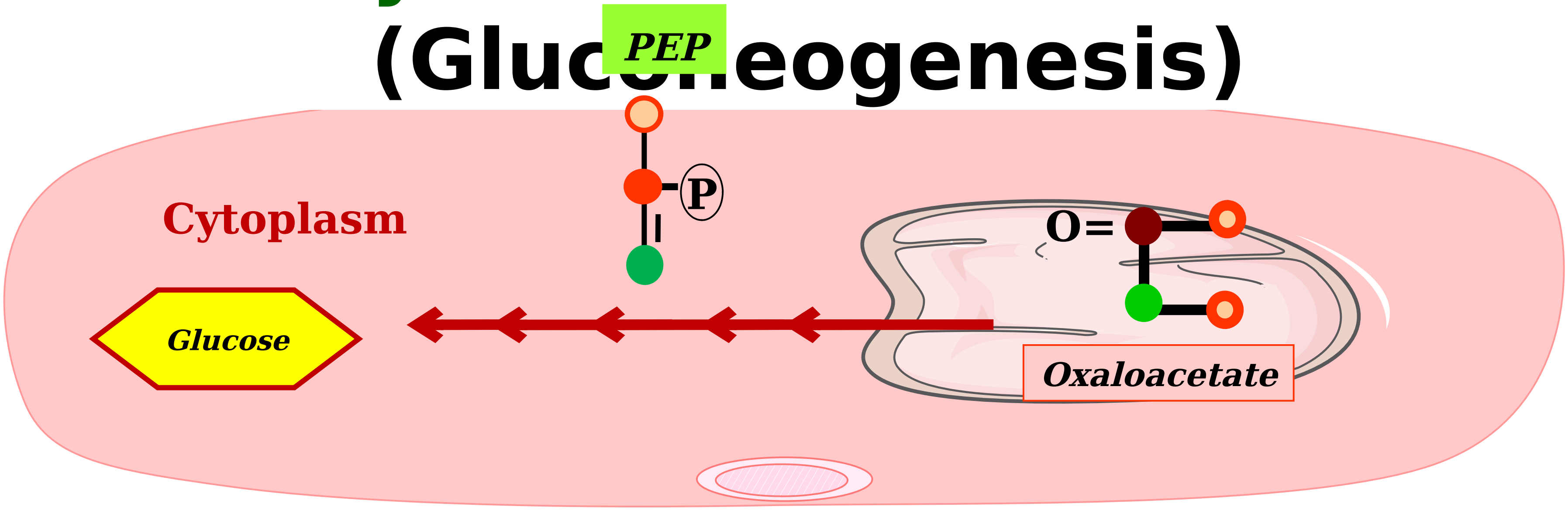


2. Non Essential a.a Synthes

(Transamination)



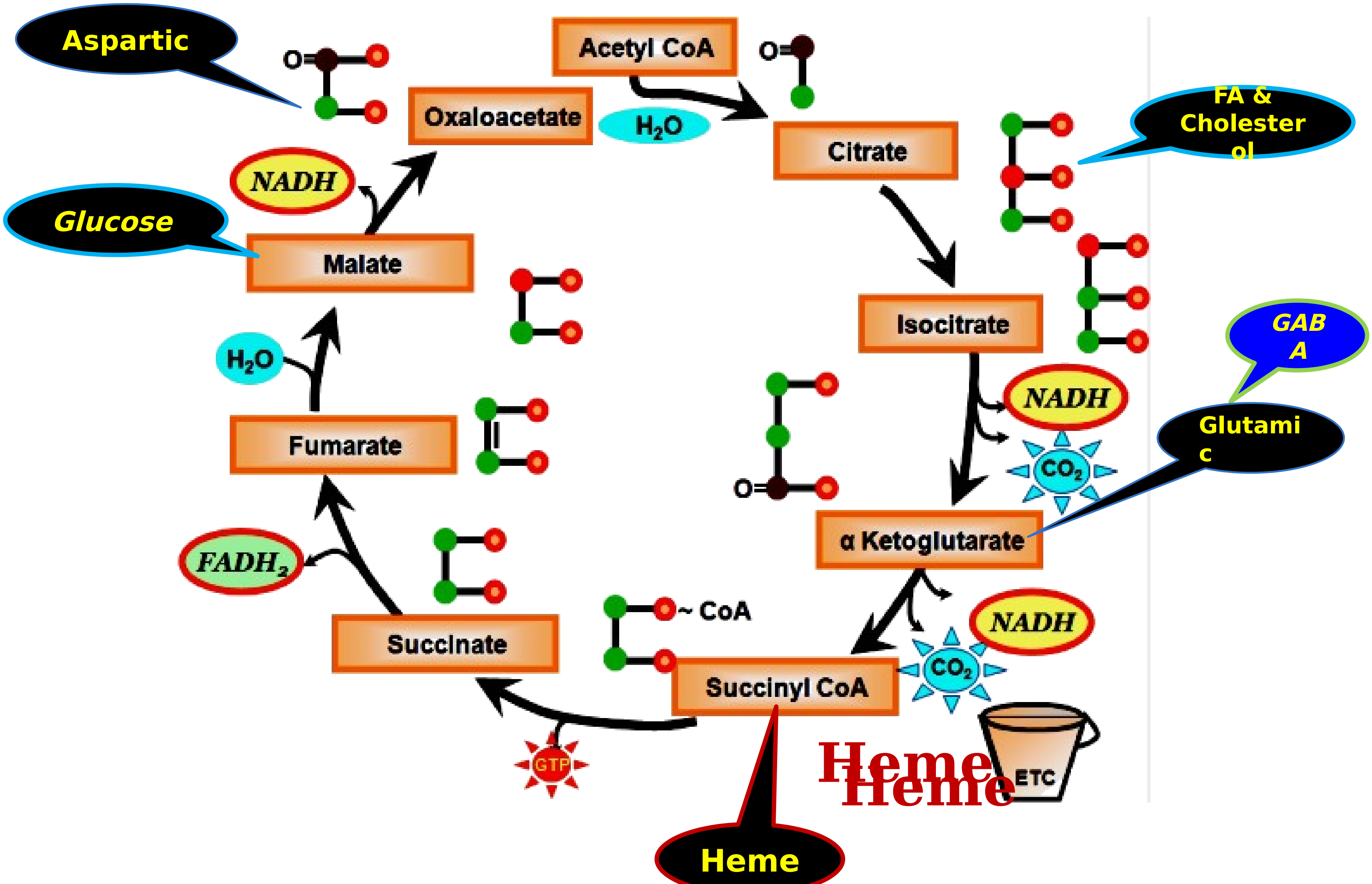
3. Synthesis Of Glucose (Gluconeogenesis)



4.Synthesis Of Heme

Succinyl COA → → Heme

Efflux Of TCA Cycle Intermediates“Divergent ”



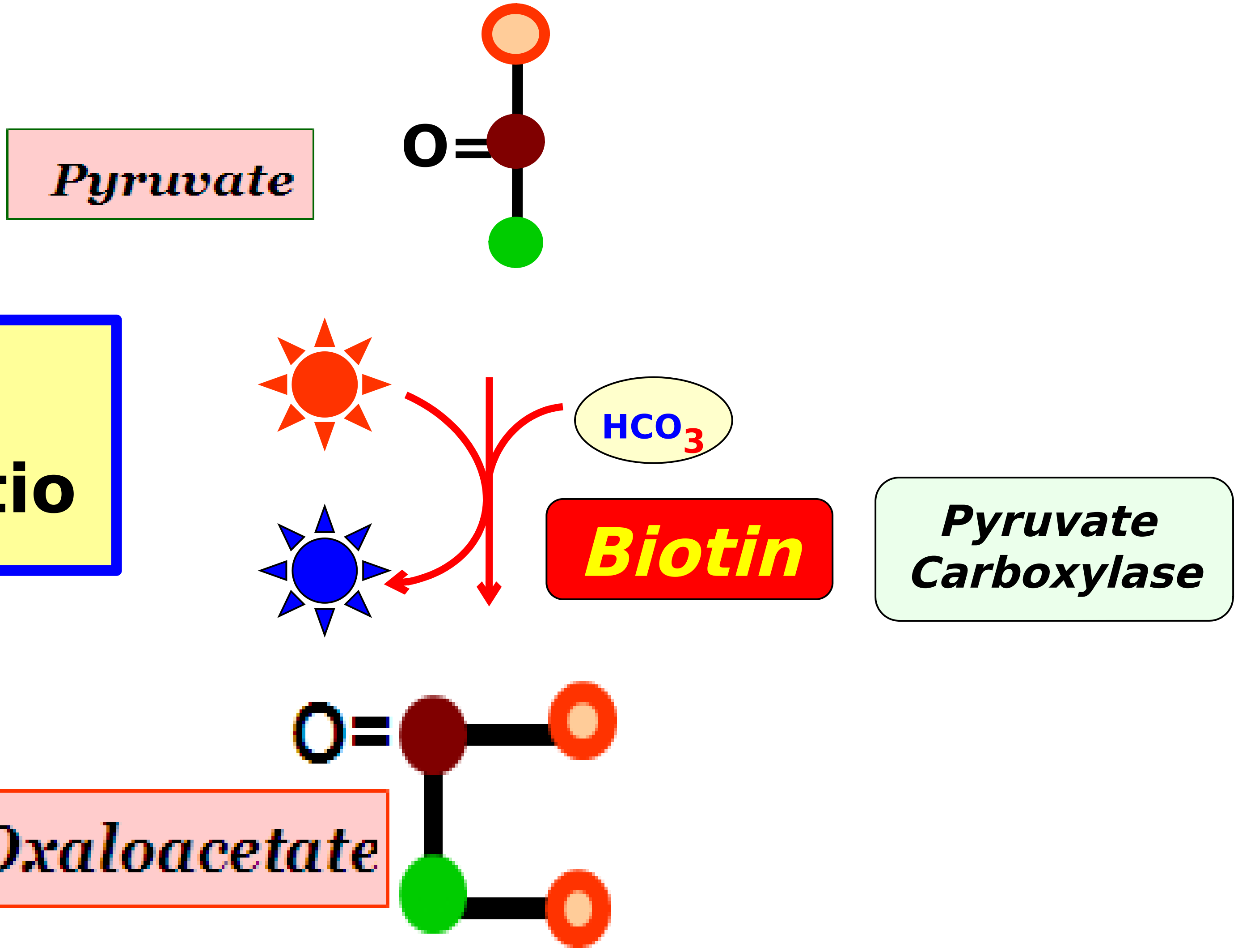
Anaploretic Reactions which replenish intermediates of TCA Cycle??

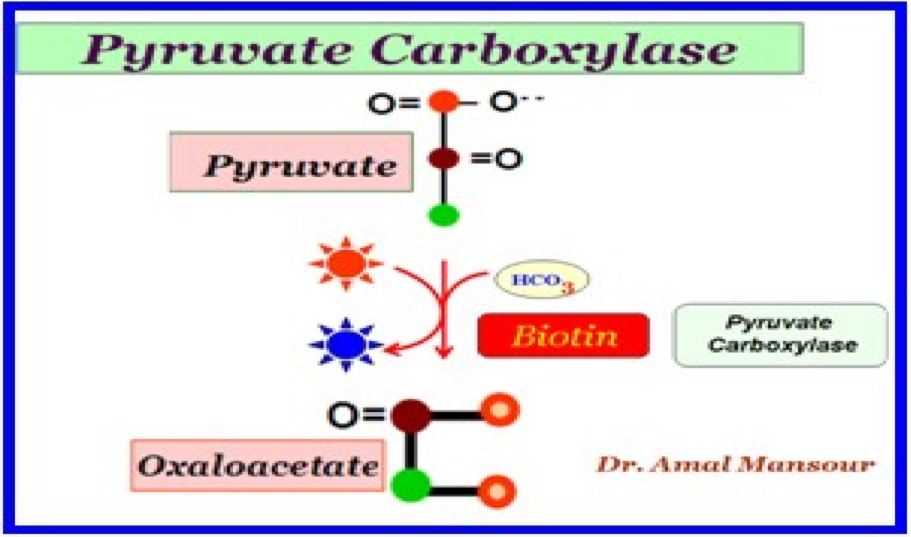
= Substrates Of TCA Cycle

Since many intermediates of TCA cycle can be used for biosynthesis of some important derivatives so you need to replenish the intermediates of TCA cycle by reactions which are called anaploretic reactions

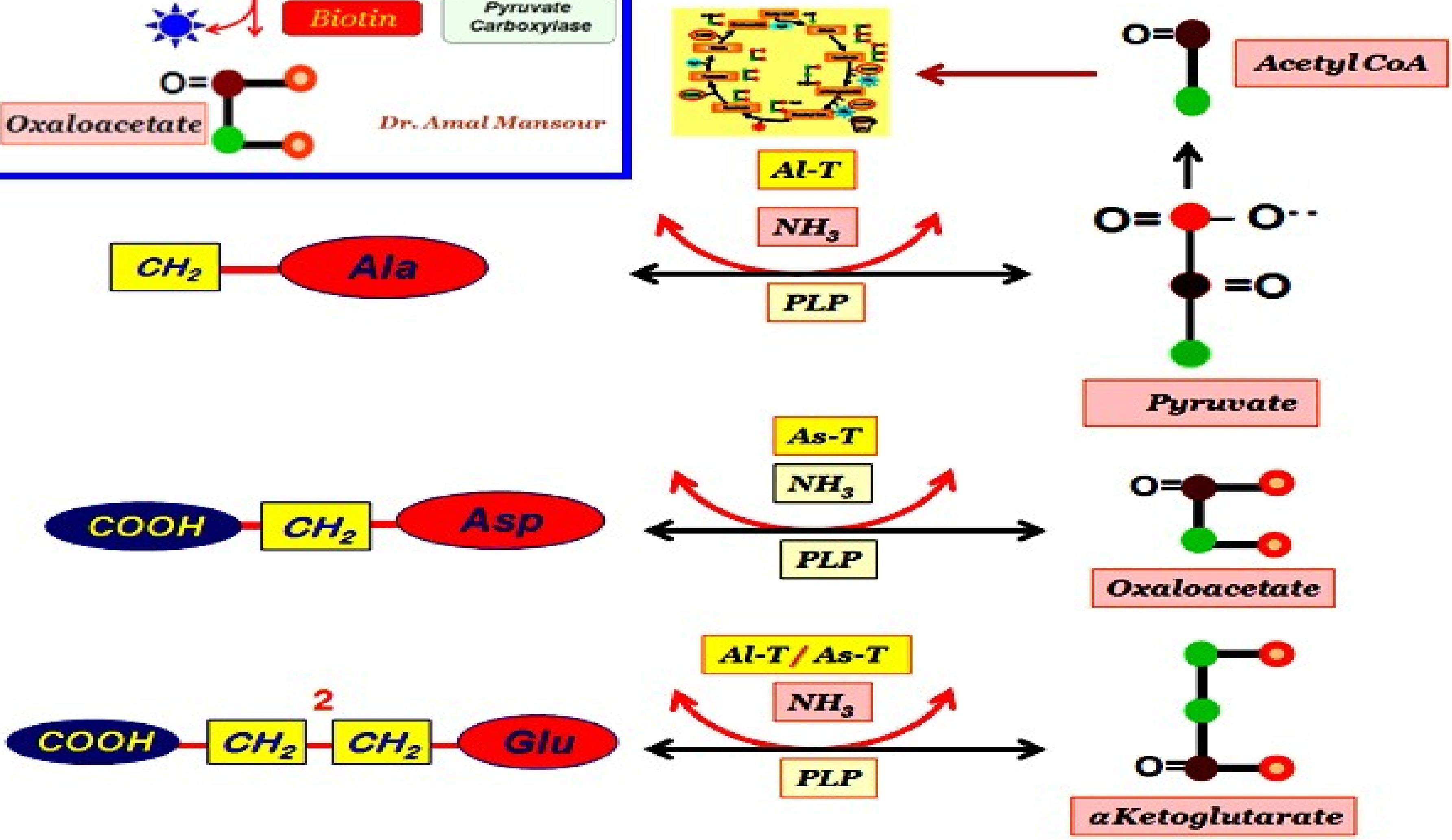
Pyruvate Carboxylase

1. Form Pyruvate Carboxylation





2. From Transamination of Non Essential a.a



Regulation of TCA cycle

(Short term)

1. Coarse Regulation (Substrate availability)

1. Low Energy State (elevated ADP & NAD^+)
2. Availability of O_2
3. Acetyl CoA & oxaloacetate

Accelerates the Rate of Reactions !!

2. Fine Regulation (Allosteric Control)

KEY REGULATORY ENZYMES

1. Citrate synthase

* Allosteric inhibitors: NADH, citrate & succinyl CoA

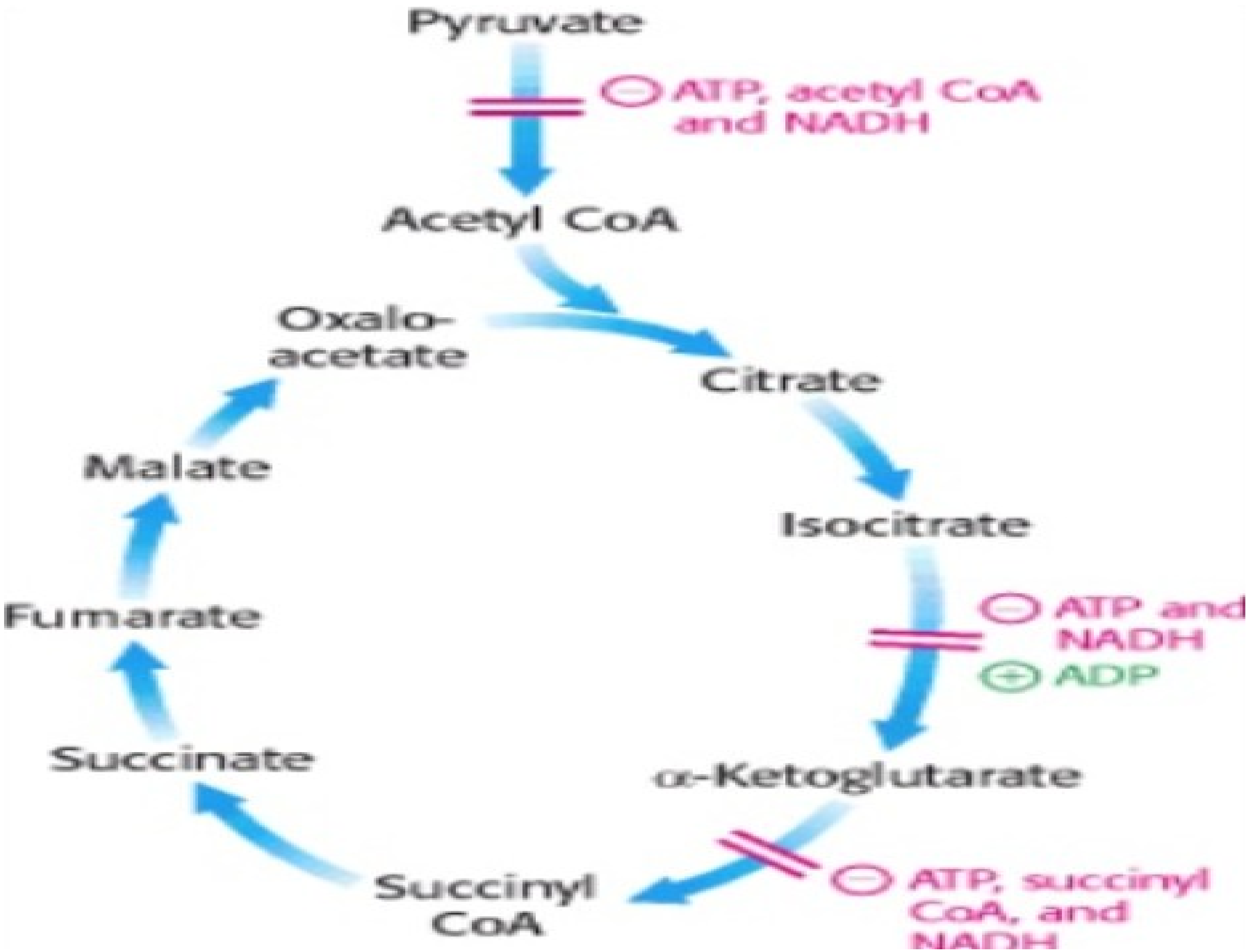
2. Isocitrate dehydrogenase

- Allosteric inhibitors: NADH and ATP
- Allosteric activators: ADP and calcium

3. α - ketoglutarate dehydrogenase

- Allosteric inhibitors: NADH, ATP, GTP & succinyl CoA
- Allosteric activators: NAD+, ADP and Calcium

Allosteric Regulation of TCA cycle



How is the rate of the TCA cycle linked to muscle contraction and the utilization of ATP?

When Ca^{++} increases in muscle cells, muscle contracts and all of the following will happen:

Isocitrate.D, α- ketoglutarate.D & PDH.

Allosterically Activated

by ⇈ in Ca++ & by ⇈ in ADP , NAD, & FAD.

Vitamins required for reactions of the TCA cycle

1. **Niacin** for the synthesis of NAD which is used in the isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, and malate dehydrogenase reactions.

2. **Riboflavin** for the synthesis of FAD, which is the cofactor for succinate dehydrogenase and α-ketoglutarate dehydrogenase.

3. **lipoic acid** cofactor for α-Ketoglutarate dehydrogenase.

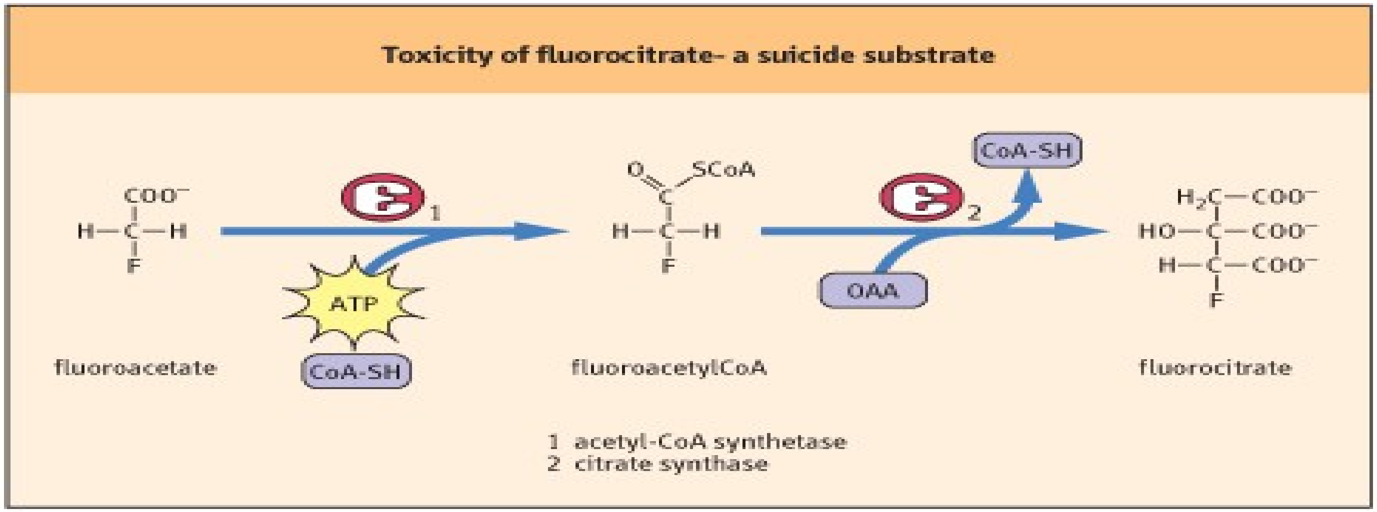
4. **Thiamine** is used for the synthesis of thiamine pyrophosphate; a cofactor for α-Ketoglutarate dehydrogenase.

5. **Pantothenate** for CoASH, a cofactor for α-

Inhibitors of TCA cycle??

1. Fluoroacetate (rat poison)

- **Give fluoroacetylCOA that condense with oxaloacetate to form fluorocitrate which inhibits aconitase enzyme**



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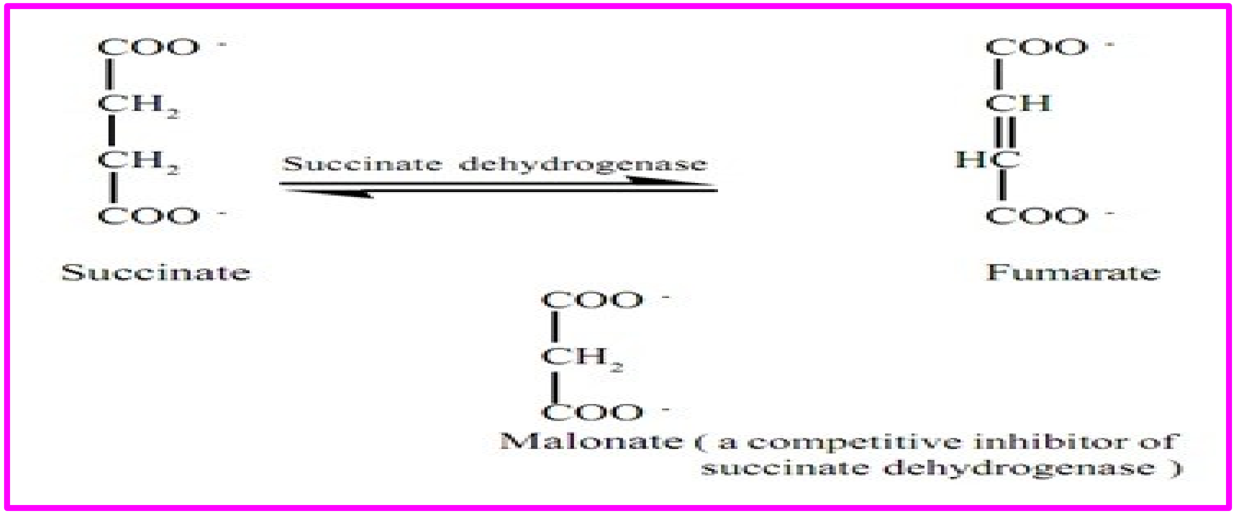
Inhibitors of TCA cycle; con..

2. Malonate

- **Similar to succinate**
- ***Inhibits Succinate dehydrogenase (competitive inhibition)***



Inhibitors of TCA cycle; con..

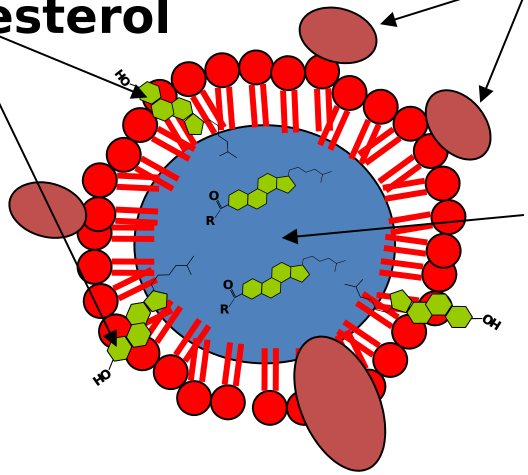
3. Arsenite poisoning:

- **Source:** Drinking water contaminated with arsenic
- **Mechanism**: inhibits enzymes and cofactors that contain adjacent free sulfhydryl groups (such as **lipoic acid**), and will interfere with TCA cycle reactions (pyruvate dehydrogenase and a-ketoglutarate dehydrogenase, it is fatal because it inhibits energy production).
- **Symptoms**: vomiting, abdominal pain, encephalopathy, and watery diarrhea that contains blood.
- **Diagnostic method**: Urine, blood, or hair testing

Lipoprotein Metabolism 1

Dr. Manal Basyouni
Professor of Medical Biochemistry & Molecular Biology

Lipid transport in the circulation



Lipids are insoluble in plasma. In order to be transported they are combined with specific proteins to form lipoproteins

Classification of Lipoproteins

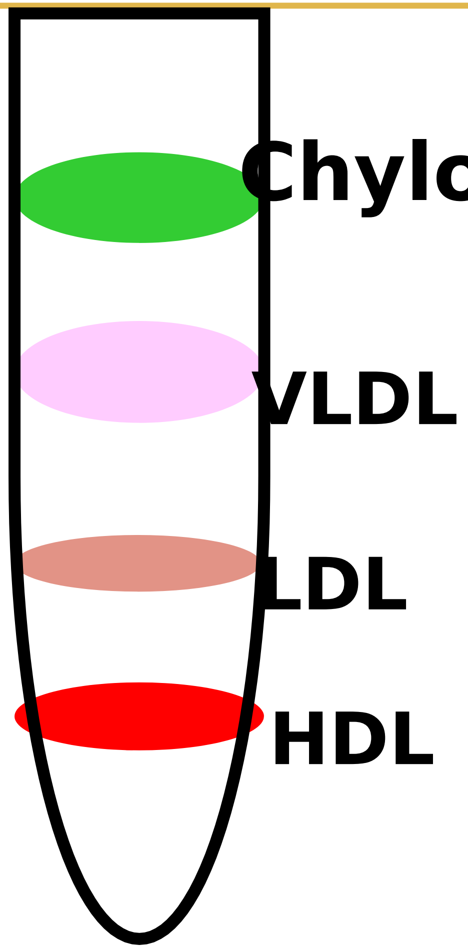
1-Chylomicrons CM: derived from small intestine and carries exogenous dietary TAG.

2-Very low density lipoproteins (VLDL) derived from liver and carries endogenous TAG.

3-Low density lipoproteins (LDL): carries cholesterol from liver to tissues (Bad Lipoprotein).

4-High density lipoproteins (HDL): carries cholesterol from tissues to liver to be excreted in bile (Good Lipoprotein).

Ultracentrifugation



- **This method separates lipoproteins according to their density.**
- **The <u>higher the fat content</u> the <u>lower the density</u> of the lipoprotein particle.**

Functions of lipoproteins

1- Transport of triacylglycerol (TAG rich particles)

Chylomicrons: transport TAG of dietary origin.

VLDL: transport TAG of endogenous hepatic origin.

2- Transport of cholesterol (cholesterol rich particles)

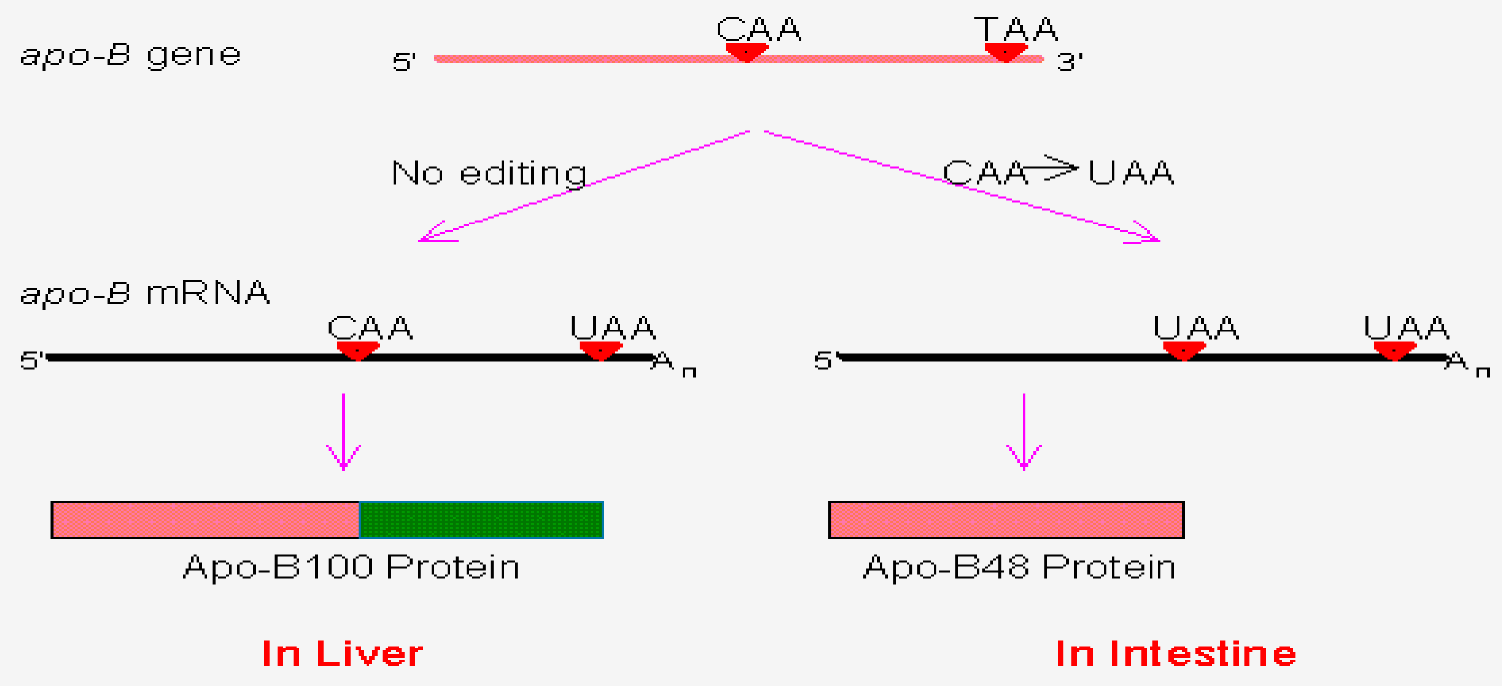
LDL: Carries cholesterol from liver to extra-hepatic tissues.

HDL: Carries cholesterol from peripheral tissues to liver.

3-Transport of fat-soluble vitamins and fat-soluble drugs.

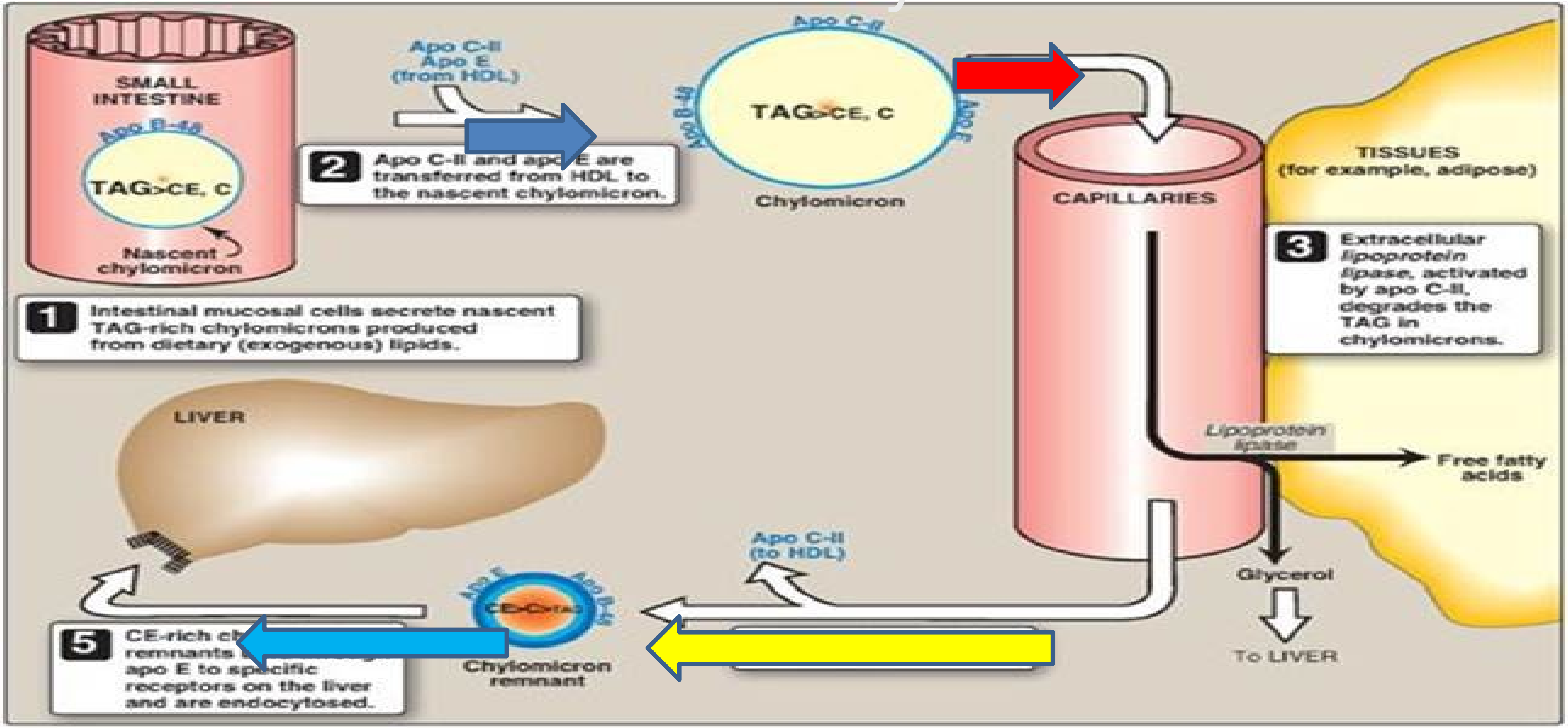
Apo B 100 Vs B48

RNA editing is a molecular process in which the base of a RNA molecule is altered by specific enzymes. In the following example, Cytosine (C) is changed to Uracil (U).



Lipoprotein Metabolism

Metabolism of Chylomicrons



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Chylomicrons (CM)

- **CM are assembled in intestinal mucosal cells , enter the lymphatics then the blood.**
- **Carry TAG of (dietary origin) to peripheral tissues.**
- **Nascent CM: contains Apo B-48**
  (48% of the protein coded by Apo B gene)
- **Mature CM : contains Apo B-48 plus Apo C-II and Apo E (taken from HDL during circulating in**

- **As CM circulate in blood, 90% of their TAG in the core are degraded by lipoprotein lipase, and decrease in size.**

- **Apo C II (but not Apo E) returns back to HDL.**

- **The remaining particles are called CM remnants, that are**

Q: What is the clearing factor?

- **Lipoprotein lipase**

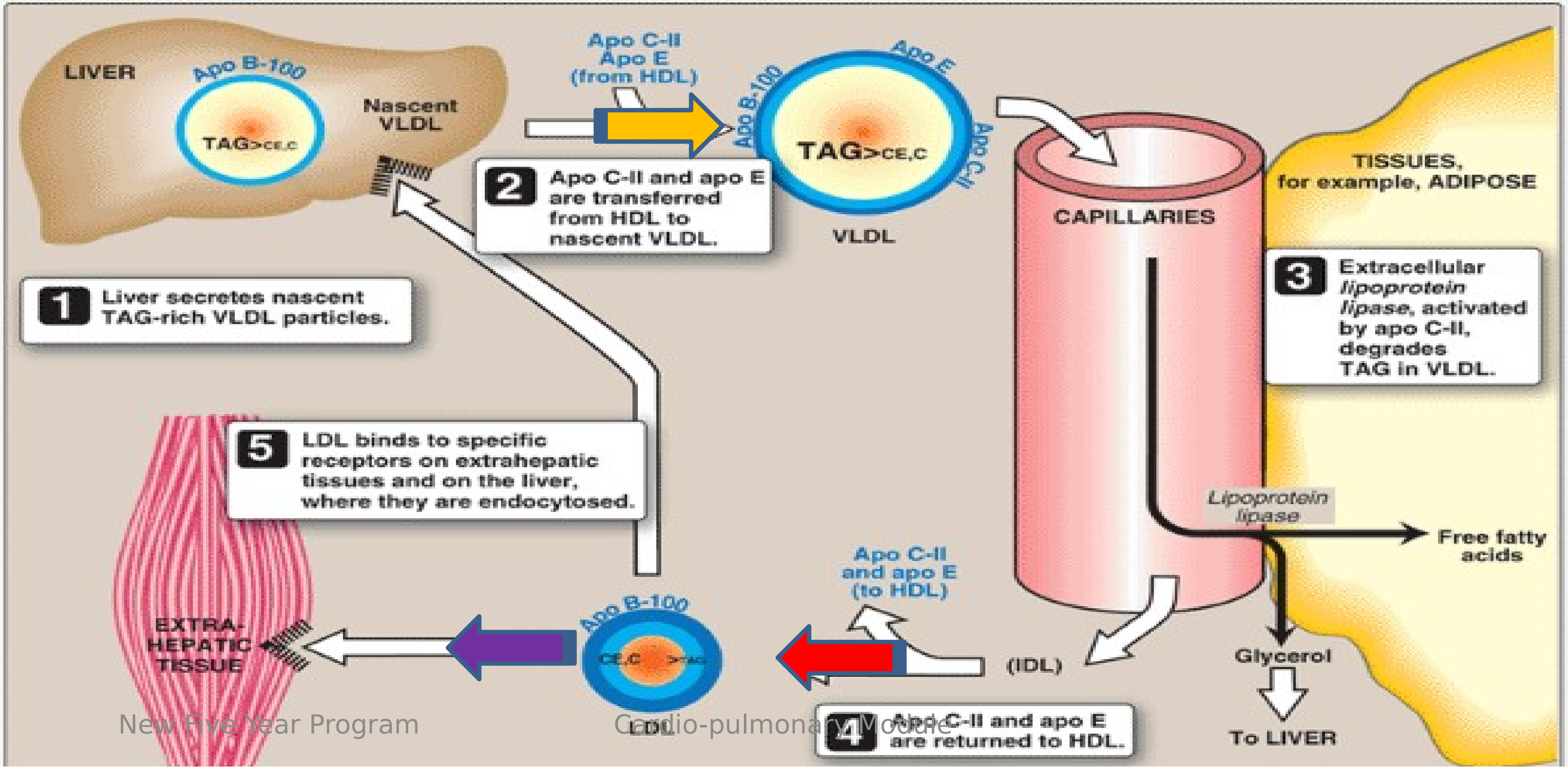
- **LPL is synthesized by adipocytes, cardiac and skeletal muscle cells, then transferred to be attached by heparan sulfate to the capillary wall of most tissues. (liver lack this enzyme)**

It hydrolyzes TAG of CM & VLDL releasing fatty acids and glycerol so it clears plasma from lipoproteins. FA are taken up by cells. Glycerol is taken by liver.

Apo CII is cofactor activating LPL.

- **Insulin enhance LPL synthesis (Induce synthesis) and activity in adipocytes (this explain why adipose cells gain fat in a well-fed state.)**
- **It is the only lipase that is induced by Insulin**

Metabolism of VLDL



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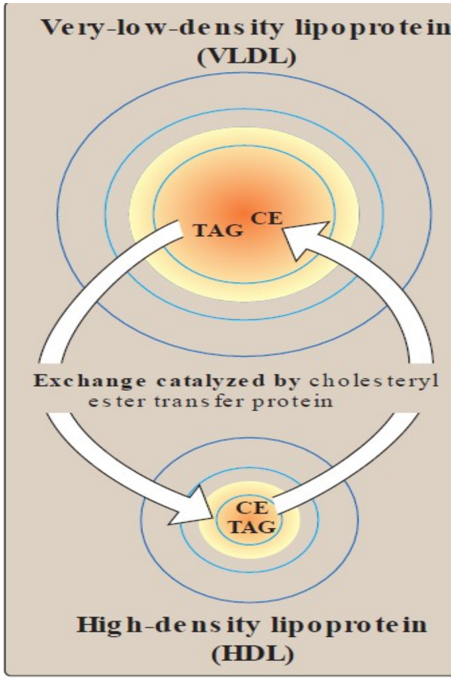
Very Low Density Lipoproteins (VLDL)

- They are assembled and secreted by the liver into the blood.
- They carry **TAG of hepatic origin** to peripheral tissues.
- **Nascent VLDL**: contains **Apo B-100** (100% of the protein coded by Apo B gene).
- **Mature VLDL**: contain Apo B-100 plus
  Apo C-II and Apo E (from HDL)
- **ApoC-II** activates **lipoprotein lipase** that degrades TAG into glycerol and fatty acids. As TAG is degraded, VLDL becomes **smaller in size and more dense.**
- Apo CII and Apo E return back to HDL
- Transfer of TAG from VLDL to HDL in exchange with Cholesterol Esters (CE) by **cholesterol ester transfer protein**
- VLDL IDL LDL



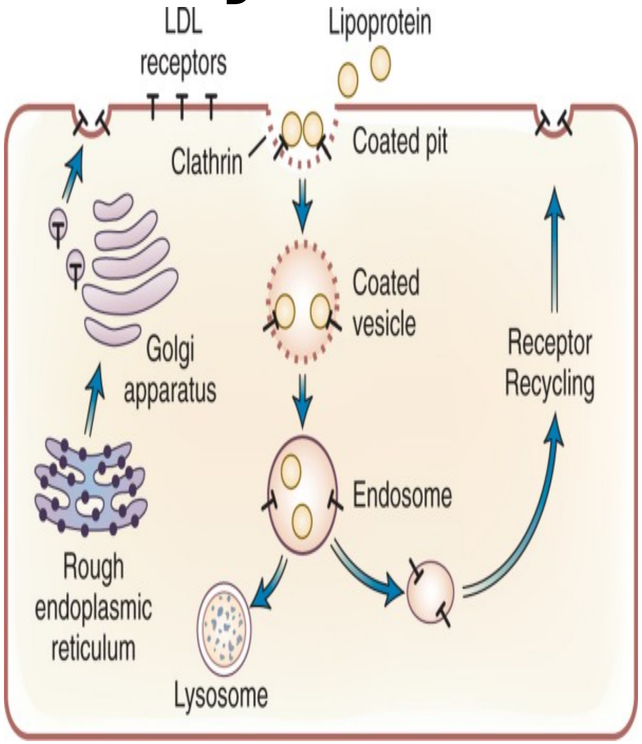
Uptake of LDL

Receptor-Mediated Endocytosis

Macrophage Scavenger Receptors

Receptor-Mediated Endocytosis

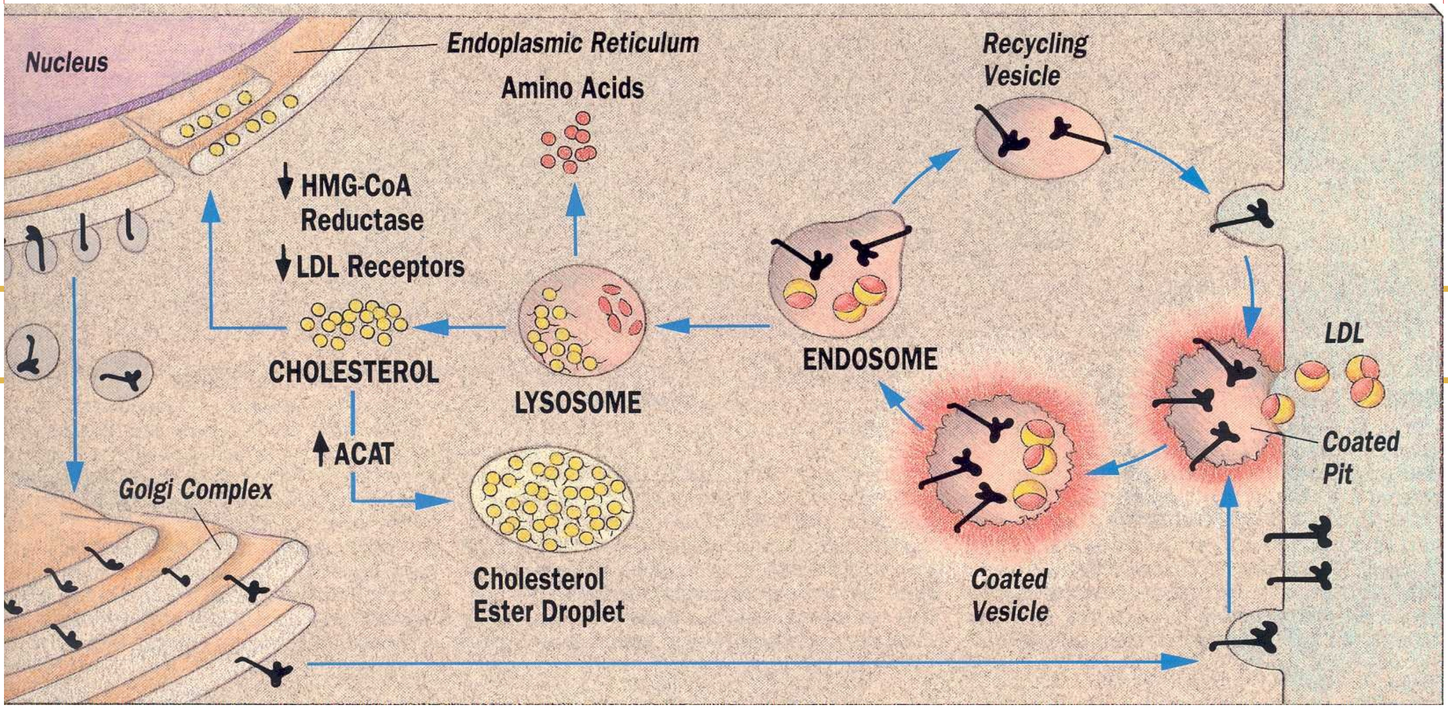
- LDL particles contain less TAG and more cholesterol ester than their VLDL precursors.
- They provide cholesterol to the tissues by binding to cell membrane LDL receptors (in both liver and tissues (that recognize Apo B -100) by receptor mediated endocytosis. (which is highly specific and regulated).
- Another method of uptake of chemically modified LDL is by machrophage scavenger receptors (not regulated).



Receptor-Mediated Endocytosis

- **LDL receptors** are –ve charged glycoproteins that are clustered in pits on cell membrane coated with the protein **clathrin** that stabilizes the shape of the pit.
- **LDL-receptor complex is endocytosed inside the cells.**
- **The vesicle containing LDL rapidly loses its clathrin coat and fuses with other similar vesicles forming endosomes.**
- **LDL receptors are recycled to cell membrane, whereas LDL in the vesicle are transferred to lysosomes where they are degraded by lysosomal enzymes releasing: Free Cholesterol, Fatty Acids, Amino acids. All are utilized by the cell.**
- **Excess cholesterol inhibits synthesis of new LDL receptors protein thus limiting further entry of LDL (down regulation of the receptor).**

Effects of Endocytosed Cholesterol on Intracellular Homeostasis



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Defects in cellular LDL receptors

Decrease in cellular uptake of cholesterol

Increase of the blood levels of cholesterol (extracellular)

Decrease intracellular levels of cholesterol

No feedback inhibition exerted on HMG-CoA

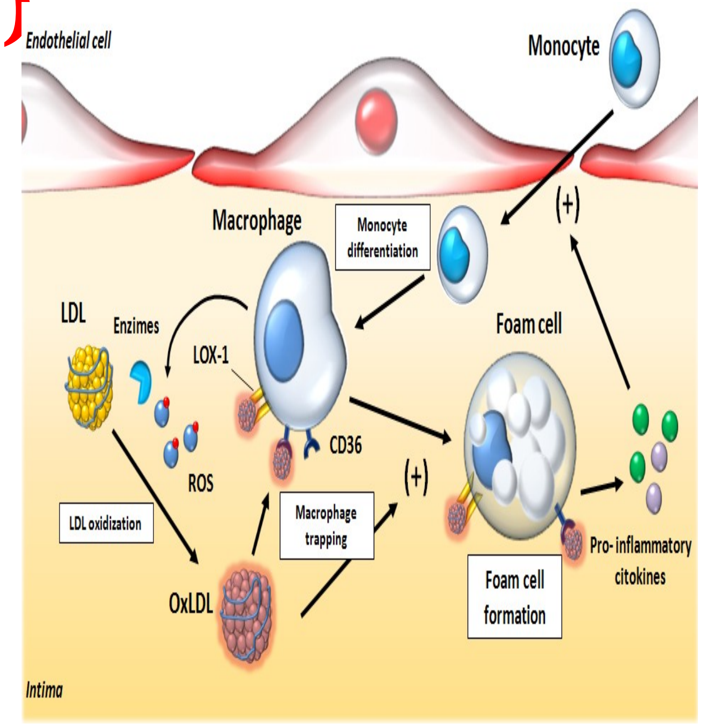
Continuous intracellular synthesis of cholesterol

More increase in extra cellular Cholesterol

Macrophage Scavenger Receptors

Oxidation of LDL (ox LDL)

- LDL can be oxidatively damaged: PUFA's are oxidized and trigger oxidation of apoB_{100} protein --> ox LDL.
- Ox LDL is engulfed by scavenger receptors of macrophages in sub-endothelial space which are not down regulated in response to increased intracellular cholesterol.
- CE accumulate in these cells and cause their transformation into foam cells.
- Foam cells accumulate in sub-endothelial layer and lead to the formation of atherosclerotic plaque. This leads to narrowing of blood vessels and predispose to thrombosis with further ischemia and infarction.



HDL Metabolism

- HDL is the main transport of cholesterol from peripheral tissues to the liver **(reverse cholesterol transport)** to be excreted through bile.

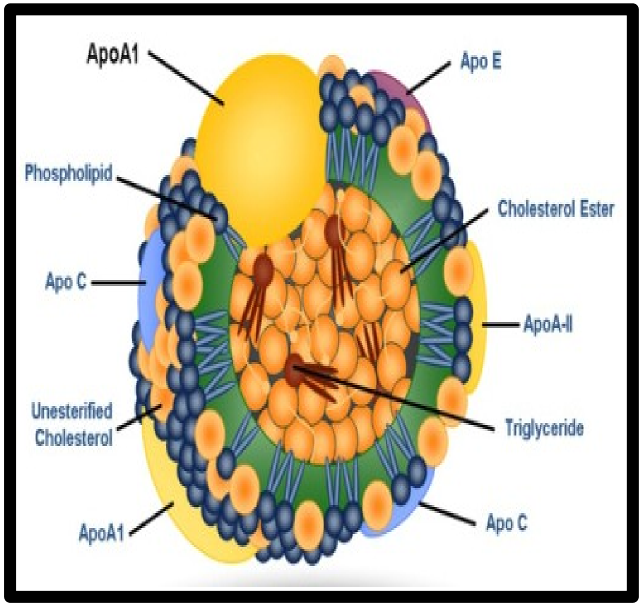
- The concentration of HDL in serum is **inversely** related to incidence of **myocardial infarction**.

- **Nascent HDL** are disc-shaped particles containing

PL mainly phosphatidyl choline and apoproteins **A-1 , C-II** and **E.**

Apo -1 constitutes about **70%** of apoproteins in HDL.

- They are made by liver and intestine and secreted in blood.



- **They are rapidly converted to spherical particles as they accept cholesterol from membranes of tissue cells.**

- **When Cholesterol is taken up by HDL, it is immediately esterified by the plasma enzyme LCAT (Lecithin : Cholesterol Acyl Transferase).**
- **LCAT is synthesized by liver and binds to nascent HDL.**

- **LCAT transfers FA from lecithin to cholesterol producing hydrophobic cholesterol ester which is sequestrated in the core of HDL .**
- **Lecithin is converted to lysolecithin that bound to serum Albumin**

- **By time, HDL increases in size by accumulating cholesterol esters inside its core.**

- **Finally HDL is up taken by the liver cells by a cell-surface receptor protein called scavenger receptor class B type 1 "SR-B 1" .**

The major functions of HDL are:

1. **Reservoir for apolipoproteins**:(apo C-II and apo E).
2. **Uptake of unesterified cholesterol**: Nascent HDLs are discoid containing PL and apo A, C, E. As they accumulate cholesterol, they acquire a spherical shape
3. **Esterification of cholesterol**: The enzyme LCAT esterifies cholesterol once it is taken up by HDL. The enzyme is synthesized by the liver and activated by apo A-I.
4. **Reverse cholesterol transport This process involves:**

a. efflux of cholesterol from peripheral cells to HDL
b. Esterification of cholesterol by LCAT
c. Binding of CE-rich HDL (HDL2) to liver (bile) and steroidogenic cells (Steroid H)
d. Selective transfer of CE to these cells and release of CE-depleted HDL (HDL3)

ATP-binding cassette (ABC) proteins

- **ABC are transport proteins using energy (ATP hydrolysis) to transport materials in and out of cells and across intracellular compartments.**
- **The efflux of cholesterol from peripheral cells is partly mediated by ABCA1**
- **Tangier disease (very rare)**

Deficiency of ABCA1
No HDL (due to degradation of lipid-poor apo A-1)

Dyslipidemia &
Biochemical Aspect of Atherosclerosis

Dr. Manal Basyouni
Professor of Medical Biochemistry & Molecular Biology
& Molecular Biology

Hyper-lipoproteinemia

- **Primary Hyper lipoproteinemia**
- **Secondary Hyper lipoproteinemia_**

Type I Hyperlipoproteinemia

- Due to a deficiency of lipoprotein lipase (LPL) or its activator apolipoprotein CII
- Dramatic accumulation (≥1000 mg/dl) of chylomicrons in plasma (chylomicronemia)
- Usually associated with acute abdomen due to acute

Type IIa hyperlipoproteinemia or Familial hypercholesterolaemia

- **The most common form.**
- **Mutation in the [LDL receptor] or the [ApoB] gene, resulting in increase plasma LDL level & therefore, plasma choleste**
- **Characterized by ... e atherosclerosis a... for early-onset ischemic heart diseases.**

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- **Associated with the presence of**

Secondary hyperlipoproteineimia

Associated with diseases as

1. **Obesity**
2. **Obstructive Jaundice**
3. **DM**
4. **Hypothyroidism**
5. **Kidney diseases as nephrotic syndrome.**

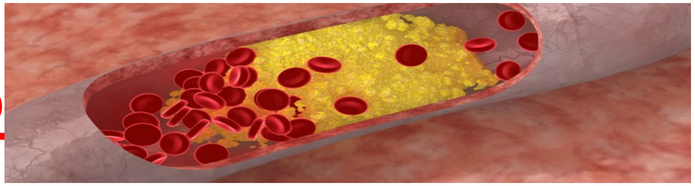
Hypolipoproteinemia

a)Tangier disease ; due to failure of the liver to synthesize Apo **A and or ABCA1 .** HDL is almost deficient from the plasma with accumulation of the CE in most tissues .

b)**Familial Abetalipoproteinemia** ; in which there is deficiency of Apo B100 . β-lipoprotein (LDL) is abscent from the plasma and most lipid fractions are of low concentration

c)**Familial hypobetalipoproteinemia** ;
This is due to decreased synthesis of Apo B100 by the liver. So the plasma level of LDL is greatly reduced .

Atheros



- **Atherosclerosis is a disease affecting arterial blood vessels.**
- **It is a chronic inflammatory response in the walls of arteries, mostly due to the accumulation of macrophages promoted by LDL without adequate removal of cholesterol by functional HDL.**
- **This leads to the formation of multiple plaques within the arteries**

Biochemical basis of development of atherosclerosis

1- Modified LDL (oxLDL: oxidized lipids and apolipoprotein) due to oxidative stress

2- Uptake of oxLDL by macrophage scavenger receptor class A (SR-A) Foam cell transformation and atherosclerotic plaque formation

Risk factors of Atherosclerosis

1. ↑Blood Cholesterol

- Diabetes mellitus
- Obstructive jaundice
- Familial hypercholesterolemia
- Hypothyroidism

Conditions decreasing Blood cholesterol

- Administration of estrogens
- Hyperthyroidism
- Hypocholesterolemic drugs (Statin)

Total Cholesterol	< 200 mg/dl
LDL-Cholesterol	< 100 mg/dl
HDL-Cholesterol	≥ 60 mg/dl
Triglycerides	< 150 mg/dl

Risk factors of Atherosclerosis

2. ↓HDL/LDL ratio ; below 0.3

↑ HDL in premenopausal women > men & postmenopausal women.

- Estrogen in women: ↓both Total cholesterol & LDL and ↑ HDL serum levels.

1. Estrogen increases expression of LDL receptors on cell surfaces, resulting in augmented clearance of LDL from the plasma.
2. It also increases HDL levels due to increase production of apolipoprotein A-I in liver.
3. Estrogen also increases formation of bile acids in liver.

3. ↓PUFA

PUFA in diet decrease the risk because it increases solubility of **cholesterol**

4. ↑Sucrose and Fructose

Sucrose and fructose are atherogenic substance tend to stimulate synthesis of triacylglycerol.

5. Coffee drinking & Cigarette smoking

- **↑ lipolysis ↑FFA ↑ plasma lipoproteins.**
- **Cigarette smoke products are oxidants and can oxidize LDL and Damages the artery wall.**
- **Cigarette smoking compromises the body's antioxidant vitamin status, especially Vit. C**

6. **Obesity and Sedentary life**

- **Increased VLDL Synthesis in liver due to increase cholesterol synthesis and lipogenesis, ↑VLDL→ ↑LDL**

7. Hypertension

- **(Blood pressure ≥140/90mmHg).**
- **Damages the artery wall allowing LDL to enter the wall more readily**

8. Family History and Age

- **Family history of premature CHD in first-degree relative**
- **Age (men ≥45 years; women ≥55 years)**

Biochemical basis of preventing atherosclerosis

1 Development of new dietary habits

- **Decrease sweets and refined carbohydrate-containing foods**
- **Restriction of cholesterol (animal fats) and saturated fat.**
- **Increase intake of healthy fats; monounsaturated, polyunsaturated fatty acids**
- **Increase intake of water soluble Fibers**
- **Increase intake of plant sterols**
- **Increase intake of Antioxidants (A,C,E)**

2 Therapeutic lifestyle changes

- Stop smoking
- Avoid stress
- Weight reduction
- Increase physical activity

3 Drug Therapy (Cholesterol lowering drugs)

- **HMG-CoA reductase inhibitors** : (statins)
- **Drugs which selectively inhibit intestinal cholesterol transporter** : (ezetimibe)
- **Bile acid binding resins:** (cholestyramine)

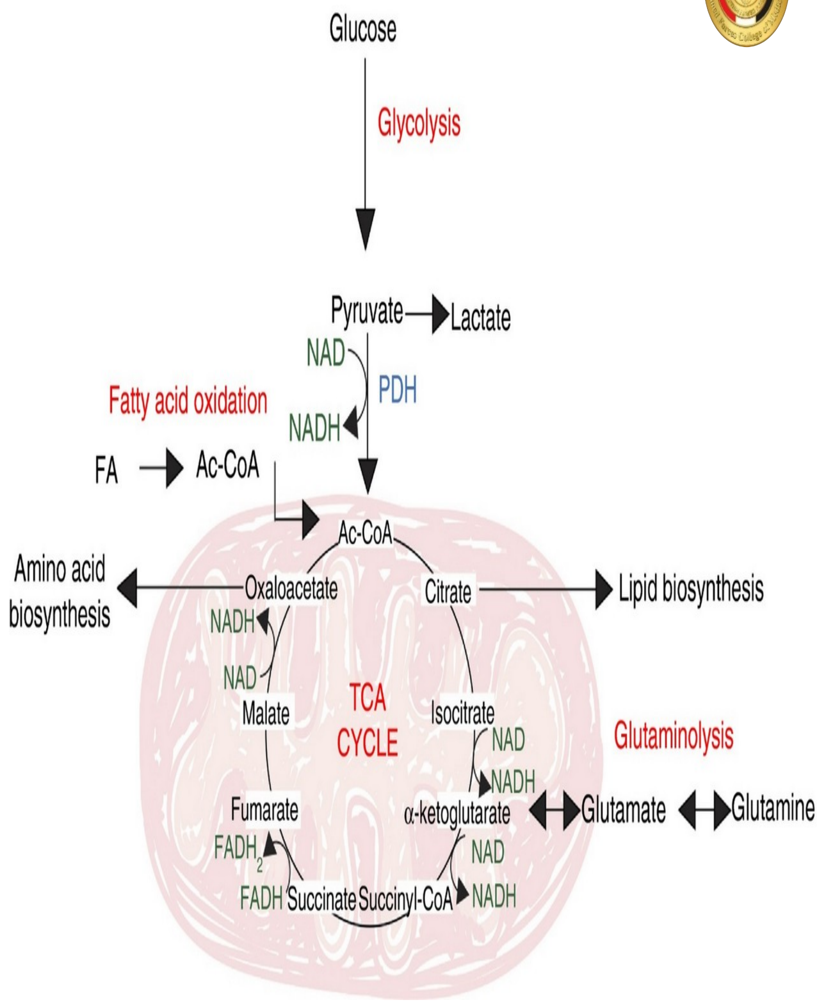
Respiratory chain (ETC) and shuttle mechanisms

By

Dr. Marwa Hamdy

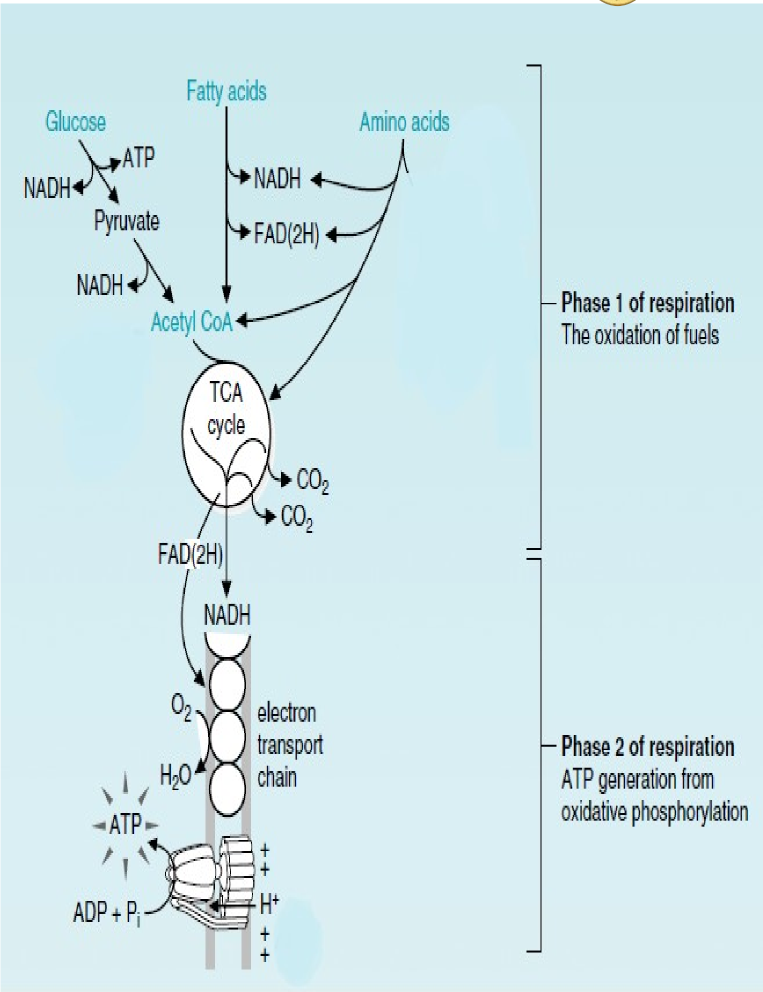
- The pathways for the **<u>oxidation of most fuels</u>** (**glucose, fatty acids, and many amino acids**) converge on the generation of **acetyl CoA**.

- \- The **complete oxidation** of the **acetyl group** to **CO2** occurs in the **tricarboxylic acid** (TCA) cycle, which collects the energy mostly as **NADH and FADH2.**



9/11/24

- The **energy derived from fuel oxidation** is converted to the high-energy phosphate bonds of **ATP** by the process of **oxidative phosphorylation** .
- - Electrons are transferred from NADH and FADH2 to O2 by the **electron transport chain** (**ETC**, located in the inner mitochondrial membrane). These series of reactions are coupled with the **synthesis of ATP**.



Fate of the NADH+H+ during Aerobic glycolysis:

- NADH produced in the cytosol cannot pass through the inner mitochondrial membrane, to reach the respiratory chain and be oxidized.

It is indirectly transferred to inside the mitochondria by what is called the shuttle mechanisms:

- ***Glycerol-3P dehydrogena[se] shuttle***
- ***Malate-Aspartate shuttle***

Many tissues contain both the glycerophosphate shuttle and the malate–aspartate shuttle

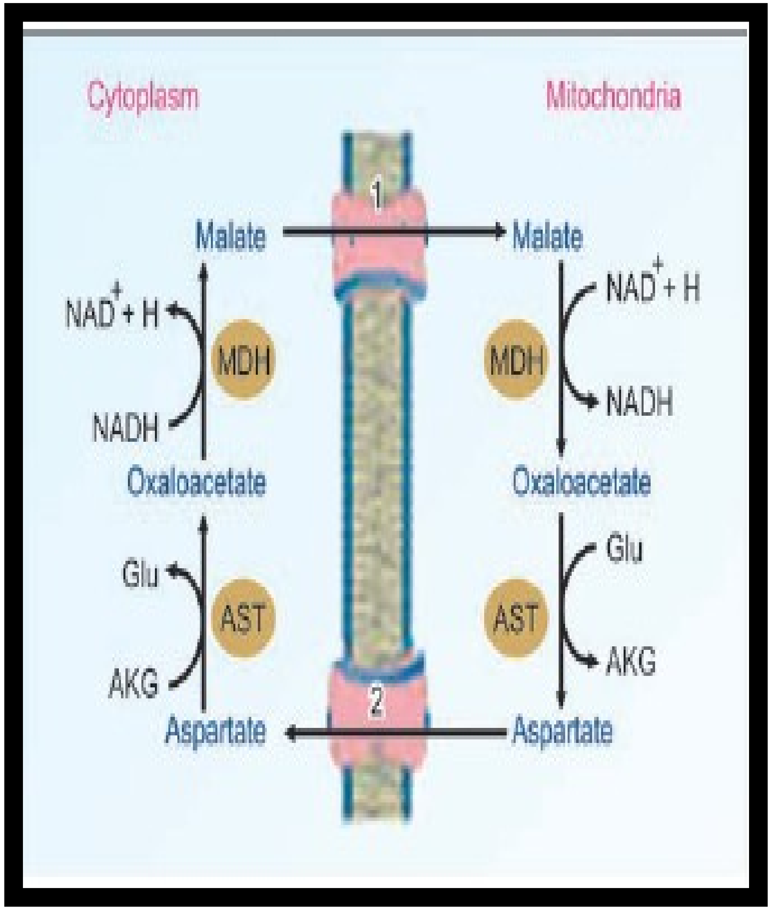
Malate Aspartate shuttle

*The cytosolic malate dehydrogenase transfers electrons from NADH to cytosolic oxaloacetate to form malate.

*Malate is transported across the inner mitochondrial membrane by a specific translocase.

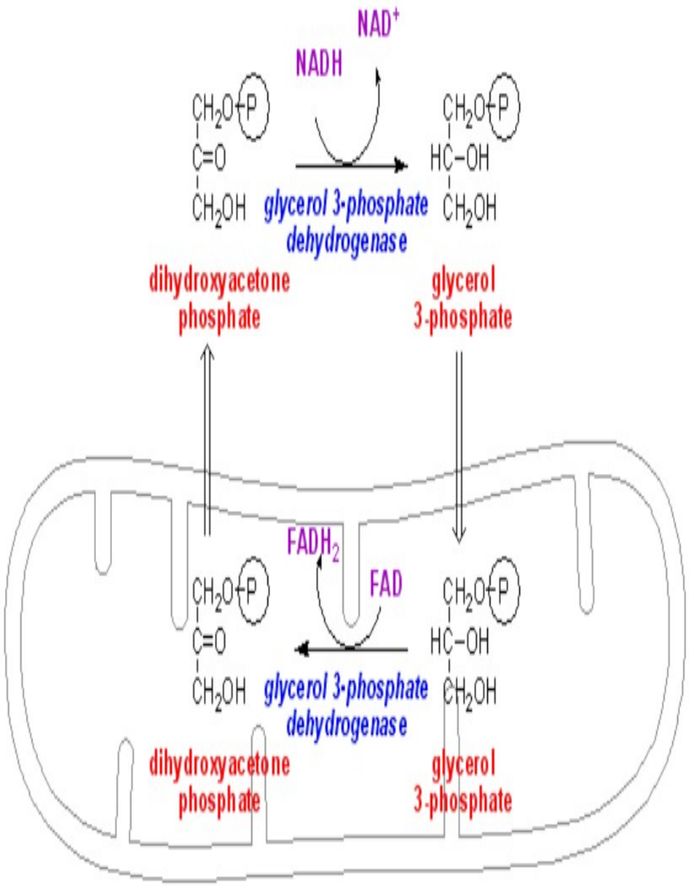
In the mitochondrial matrix, malate is oxidized back to oxaloacetate by mitochondrial malate dehydrogenase, and NADH is generated. This NADH can donate electrons to the electron transport chain with generation of approximately 3 moles of ATP per mole of NADH.

*The newly formed oxaloacetate cannot pass back through the inner mitochondrial membrane. Therefore, oxaloacetate is converted by AST into aspartate. Aspartate is, then, transported out



The Glycerol 3- Phosphate Shuttle

- The glycerolphosphate shuttle is **the major shuttle** in most tissues.
- * In this shuttle, **cytoplasmic glycerol 3-phosphate dehydrogenase** transfers electrons from **NADH** to **DHAP** to form **glycerol 3-phosphate**
- * Glycerol 3-phosphate then diffuses to the inner mitochondrial membrane.



Importance of ATP as a Source of Energy

(1) Synthesis of Macromolecules:

DNA, RNA and proteins.

Glycogen and triacylglycerol.

Structural molecules: membranes.

(2) Support the endergonic reactions in metabolic pathways.

(3) Important for active transport across membranes.

(4) Important for muscle contraction.

1. Substrate Level Phosphorylation

ADP + **A~P** → **ATP**

- Transfer of high group potential to incorporate Pi with high energy into ADP to from ATP.
- It may occur in cytosol or mitochondria.

The group transfer potential may be:

- -phosphates as 1,3 BPG, phosphoenol pyruvate “glycolysis” and creatineP "muscles".
- -Succinyl CoA, a thioester can participate in substrate level phosphorylation “TCA cycle”.

2. Oxidative Phosphorylation (Factory)

Oxidative phosphorylation, the major energy – generating process of most cells occurs in the mitochondria. The mitochondria is called the power house of the cell.

By

Elecrton Transport Chain

(Respiratory Chain)

Oxidation-Reduction

- **Oxidation**

  Addition of oxygen

  or removal of hydrogen or an <u>electron</u>

- **Reduction**

  Addition of an <u>electron</u> or hydrogen

  or removal of oxygen

Electron Transport Chain

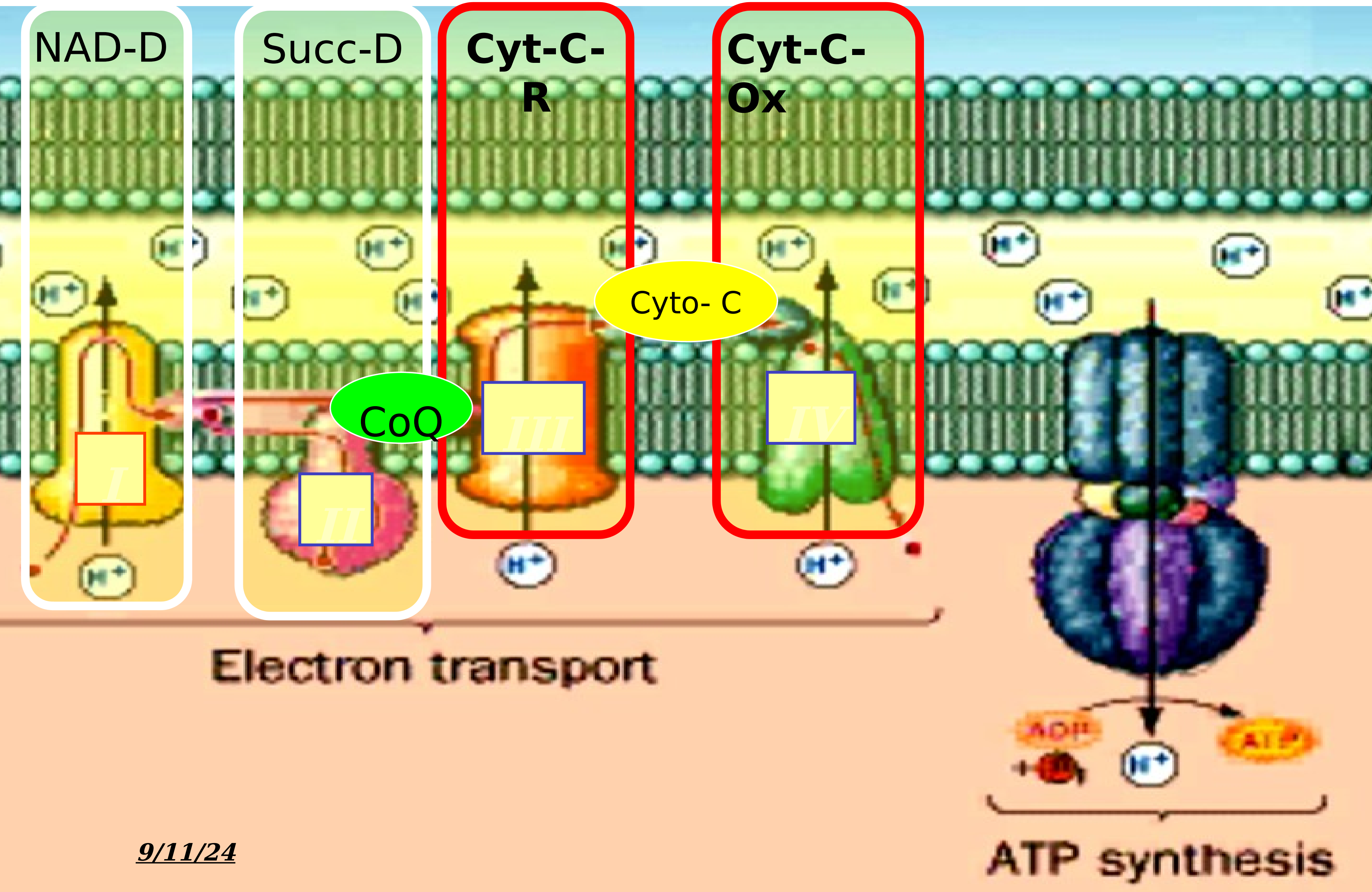
(Respiratory Chain)

Definition

It is **a sequence** of **enzymes & carriers** which are located on the **inner mitochondrial membrane.**

It is responsible for the transport of electrons from reducing equivalents (NADH and $FADH_2$) to O_2 to produce H_2O.

Also **It has the machinery** for **trapping** the liberated free energy to form ATP.


NAD-D
Succ-D
Cyt-C-R
Cyt-C-Ox
Cyto- C
CoQ
Electron transport
ATP synthesis

9/11/24

Three types of electron carriers function in the respiratory chain

(1) **Ubiquinone "Coenzyme Q"**

(2) **Cytochromes**

(3) **Iron – sulfur proteins**:

Ubiquinone "Coenzyme Q"

It is fat soluble benzoquinone (similar to vit K)

it is **freely diffusible within the lipid bilayer** of the inner mitochondrial membrane

Acts as an intermediate electron carrier

Transfers electrons from Complex I, II

→ to complex III

Cytochrome C

Cytochromes are a class of proteins that contain a heme group tightly bound to the protein.

Three types (a, b and c) participate in the respiratory chain.

All are integral membrane proteins with exception of cyt c, a soluble free protein

Cytochrome c is an Intermediate electron carrier Between Complex III & IV

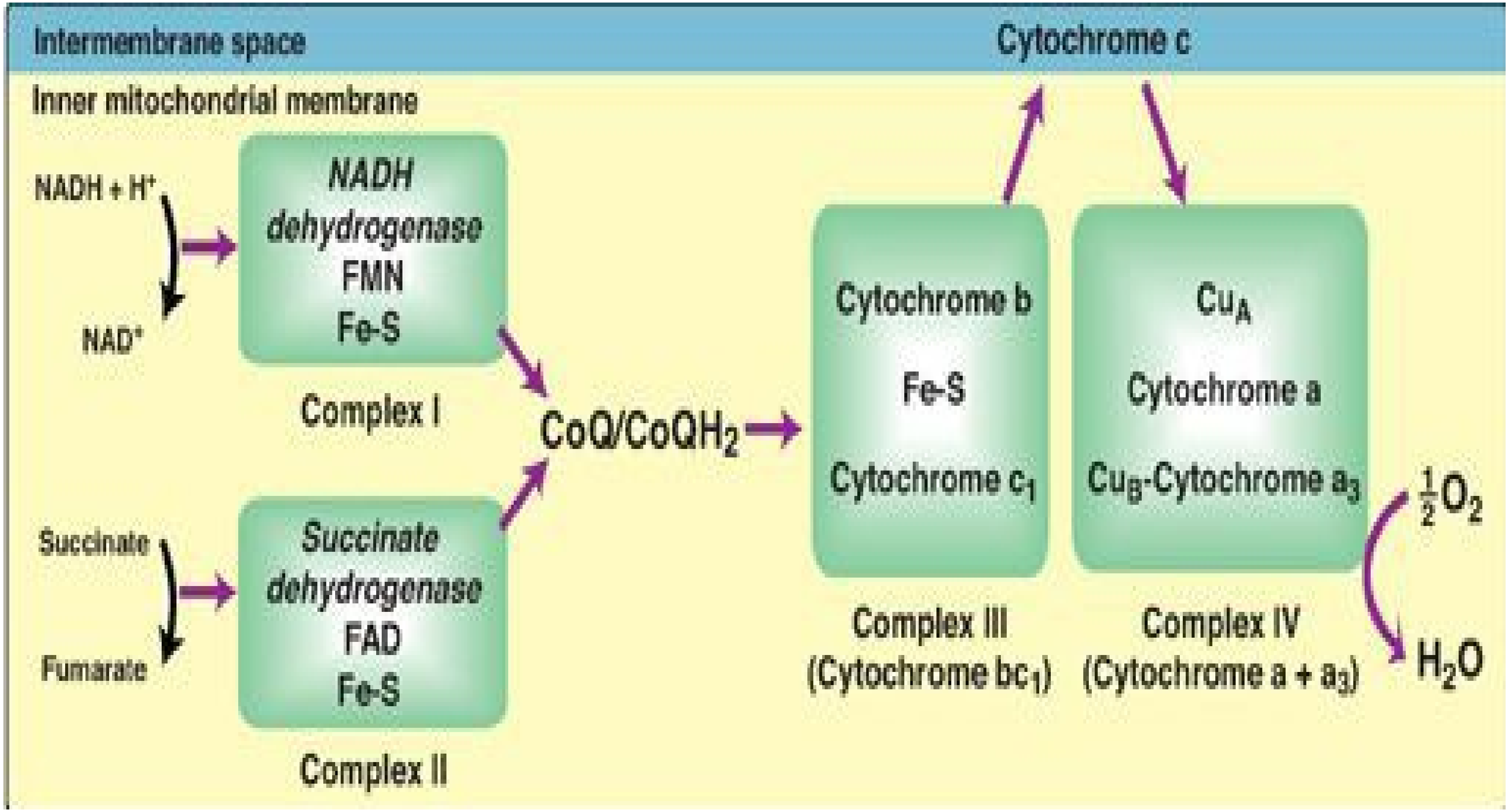
Iron – sulfur proteins

Contrary to cytochromes, iron is not present in heme but in association with inorganic sulfur atoms or with sulfur atoms of cysteine residues in the protein. Iron sulfur proteins participate in one electron transfer.

ETC components are arranged in the inner mitochondrial membrane forming 5 complexes

- **Complex I= NADH Dehydrogenase**
- **Complex II= Succinate Dehydrogenase**
- **Complex III= Cytochrome C Reductase** (b-c_1)
- **Complex IV= Cytochrome C Oxidase** (a-a_3)
- **Complex V= ATP synthase Complex**

Respiratory chain consist of enzymatic complexes



Arrangments

The major components of the **ETC** are arranged **sequentially** in an order so that the electrons flow through the chain in a **stepwise manner** from the **e^- donors molecules** to the **e^- acceptor (with high e^- affinity) i.e. oxygen.**

This movement of electrons releases energy **used in ATP synthesis.**

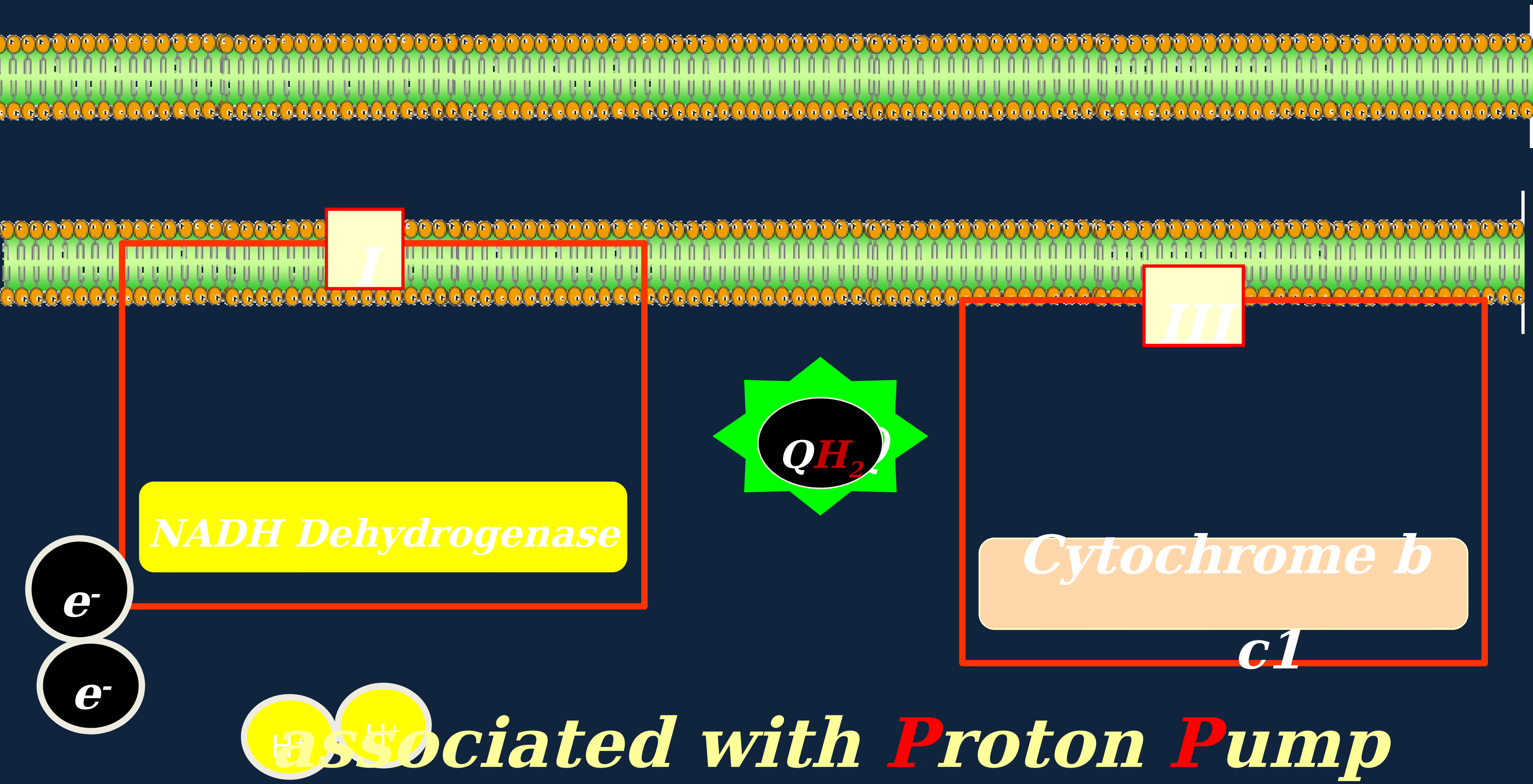
Complex I

"NADH dehydrogenase" It collects the ***NADH*** & associated with **CoQ.**

oxidation of one NADH and transfer of two electrons to ubiqinone

- During oxidation of one NADH and transfer of two electrons to ubiqinone by complex I, two protons are pumped across the mitochondrial membrane from the matrix side (N for negative face) to the cytosolic side (P for positive face). The energy released during oxidative reactions occuring in complex I is conserved by concomitant pumping of protons across membrane.

Complex I & Co Q



associated with Proton Pump

Complex II

"Succinate Dehydrogenase" It is associated with CoQ

It is the **entry point of $FADH_2$**

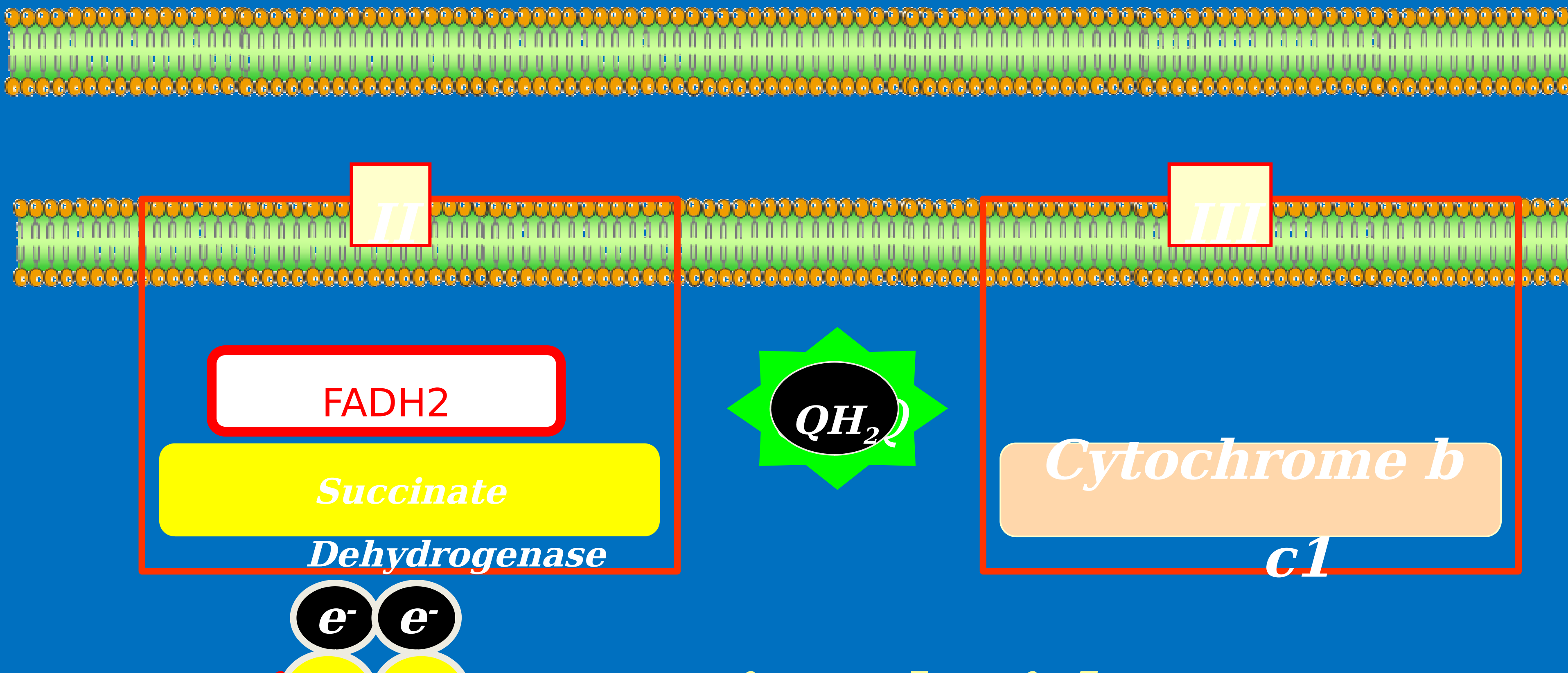
the transfer of electrons

from **$FADH_2$** to CoQ

It is Not associated with Proton Pump !!

Because It does Not ***traverse the whole length of the inner mitochondrial membrane***.

Complex II & Co Q



It is Not associated with Proton Pump !!

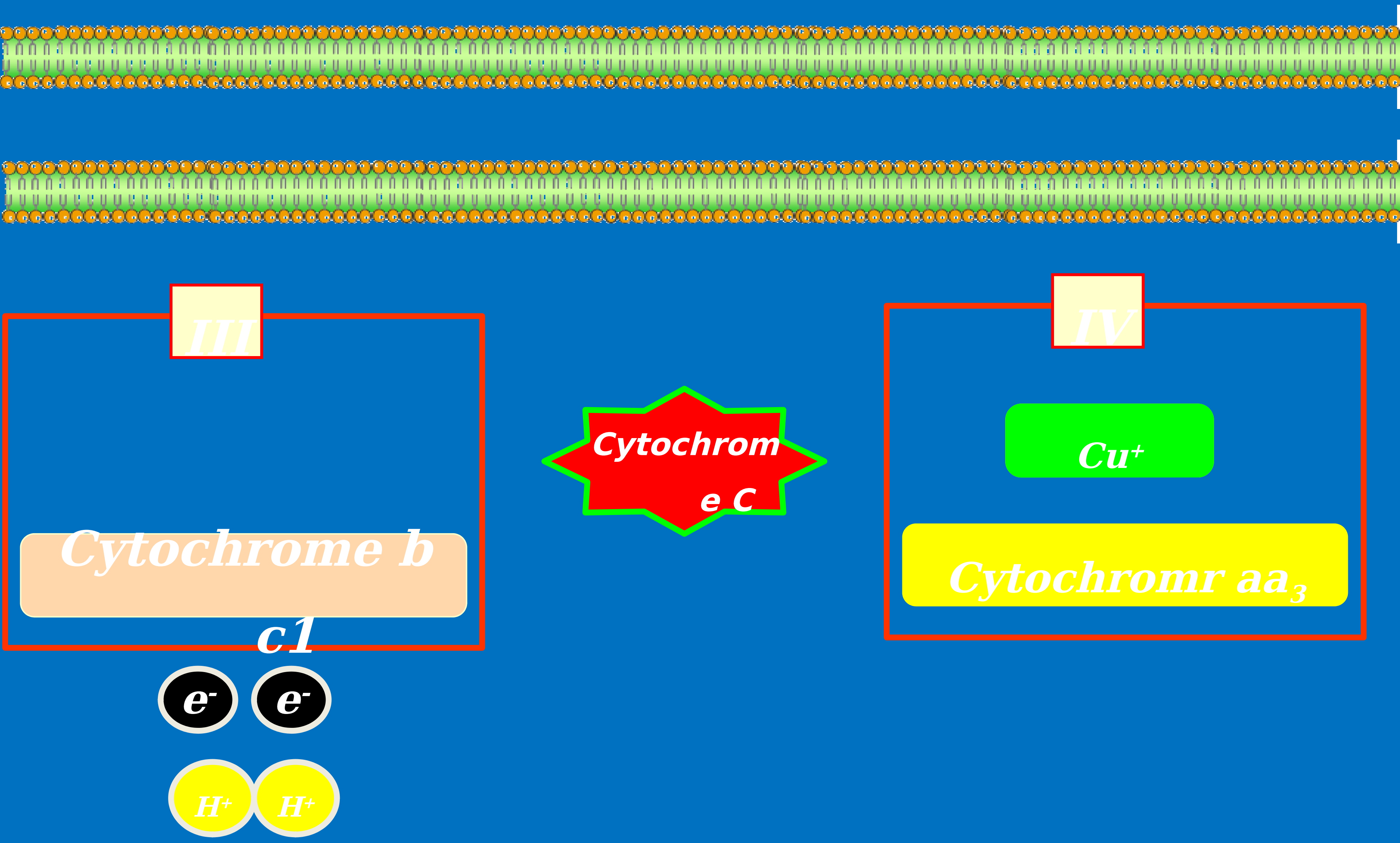
Complex III

Cytochrome C reductase

"b-c_1 Cytochrome Complex"

It transfers electron from QH2 to cytochrome C. Pumping of protons across the inner mitochondrial membrane occurs.

associated with Proton Pump !!


III
IV
Cytochrom
e C
Cu+
Cytochrome b
c1
Cytochromr aa3
e-
e-
H+
H+

Complex IV "cytochrome C oxidase complex"

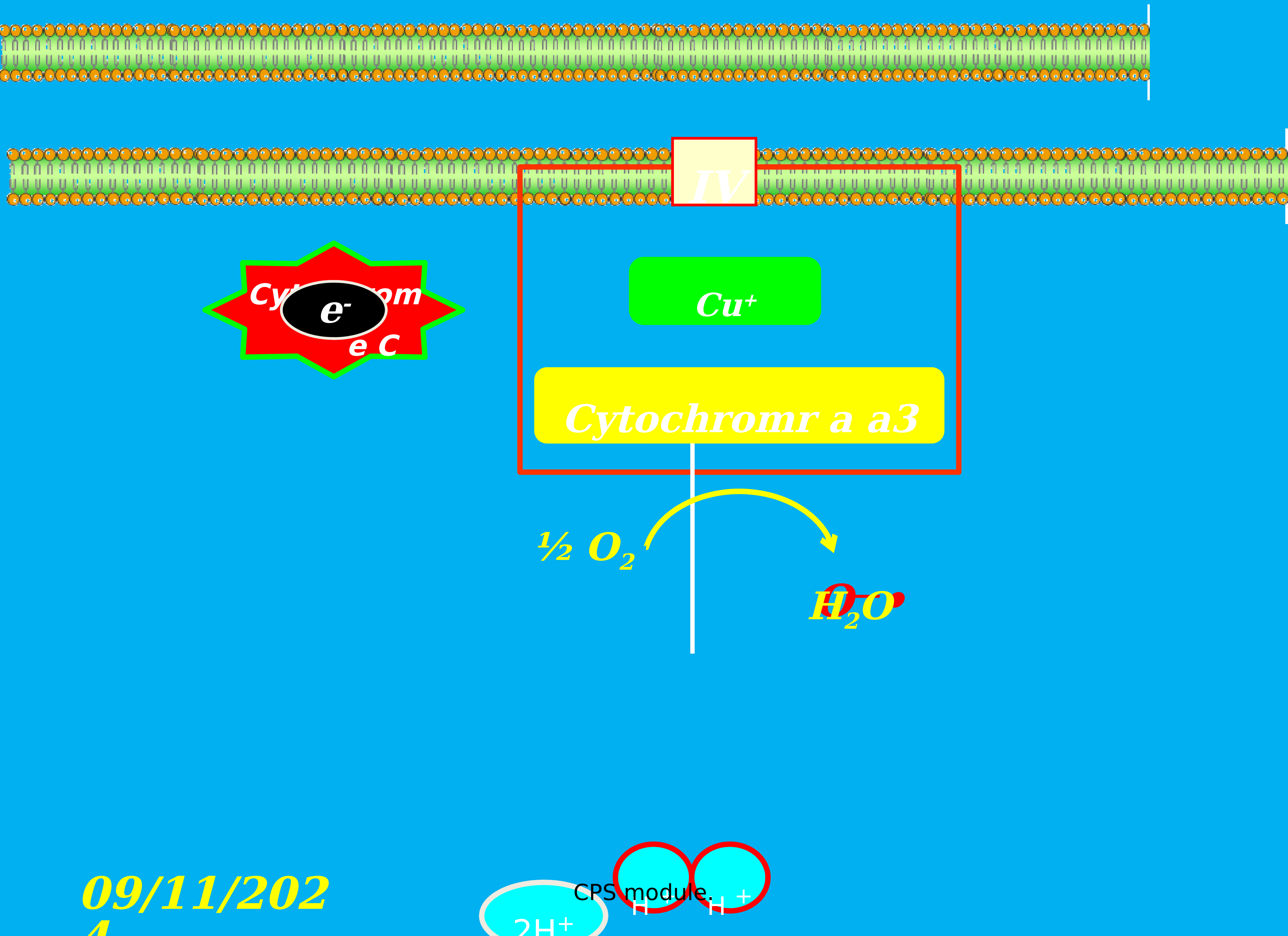
It is a large enzyme (**Cytochrome aa$_3$**)

It contains

2 heme groups "cyt **a** & **cyt a$_3$**"

2 Cu ions

catalyzes the transfer of electrons from reduced cyt c to molecular oxygen associated with Proton Pump !!


e-
IV
Cu+
Cytochromr a a3
½ O2
H2O
2H+
H+
H+
CPS module.

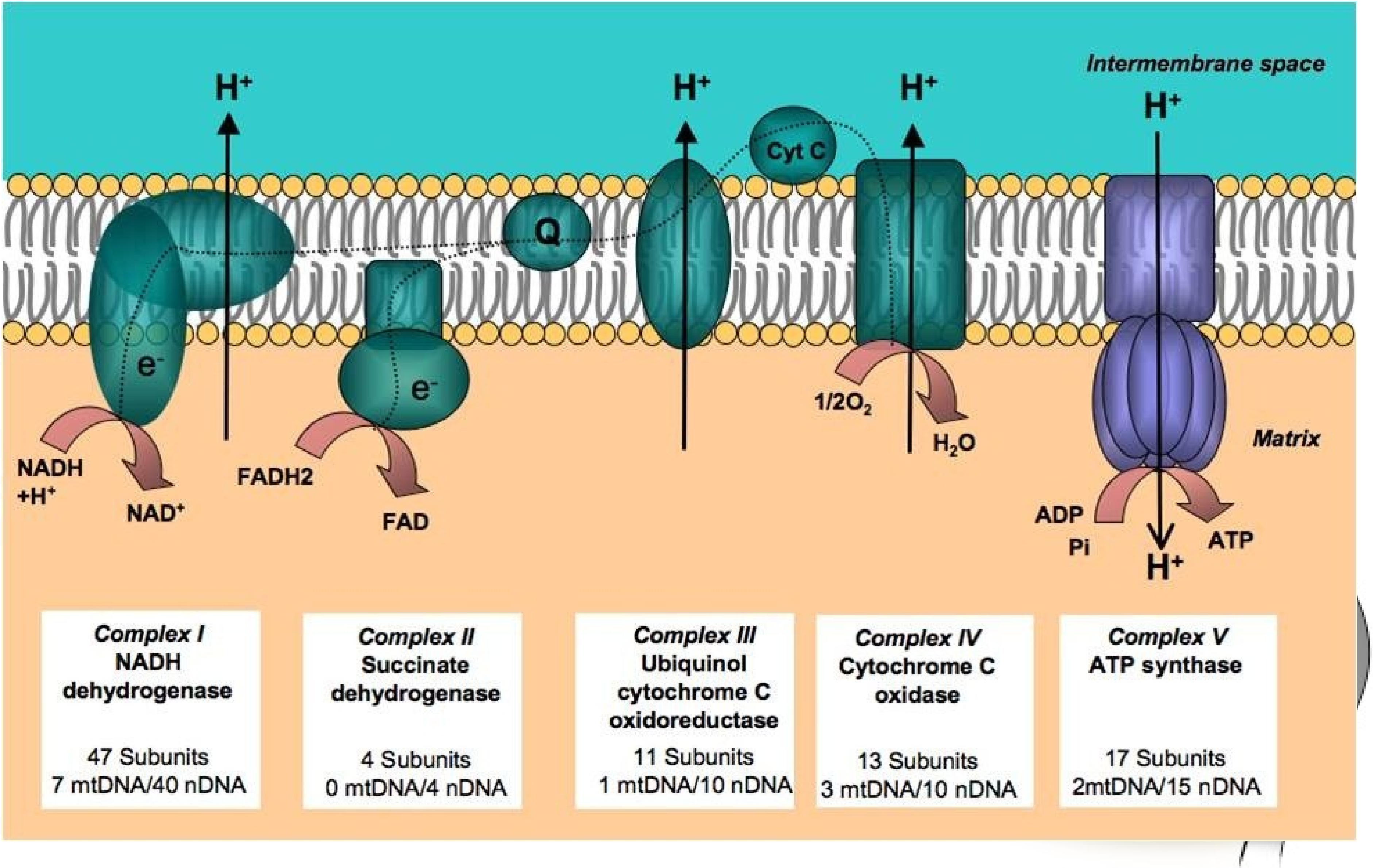
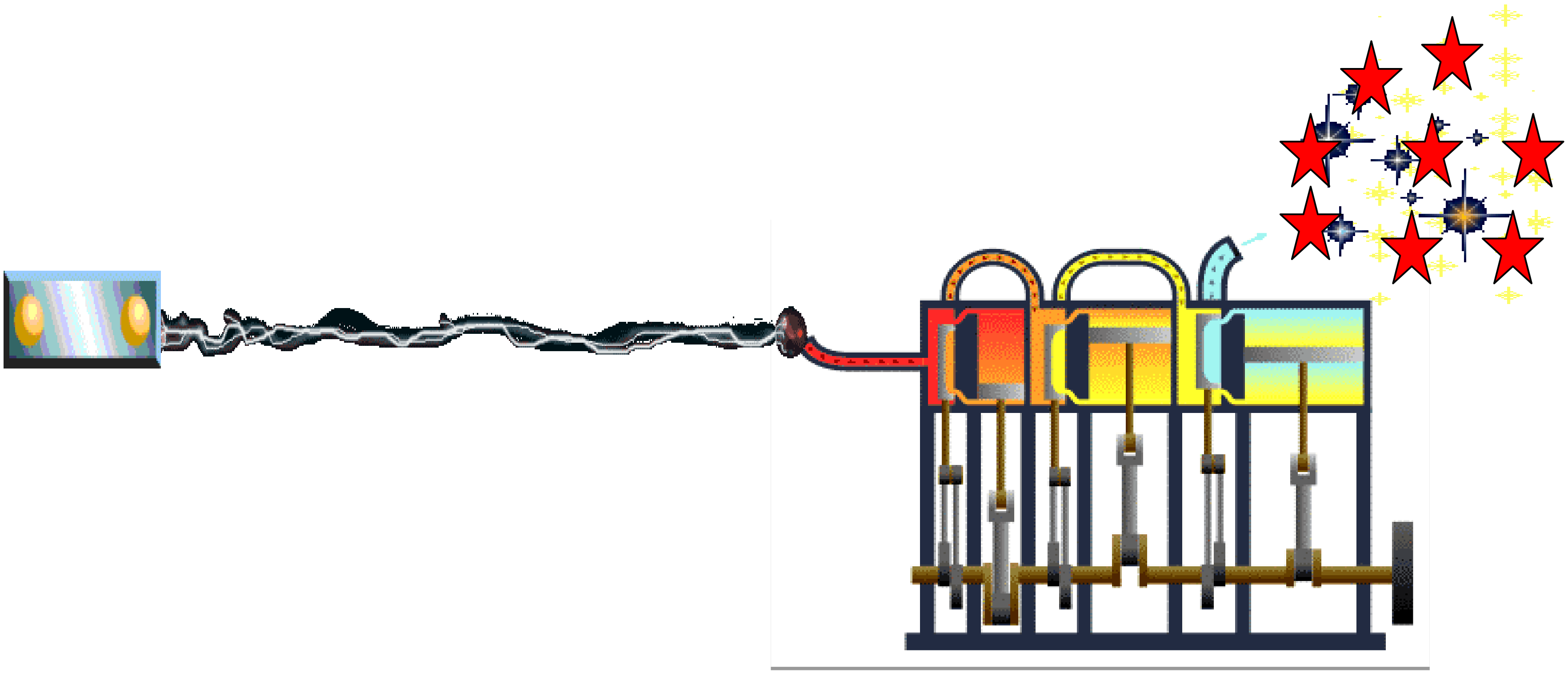


Figure 14-7 Essential Cell Biology, 2/e. (© 2004 Garland Science)

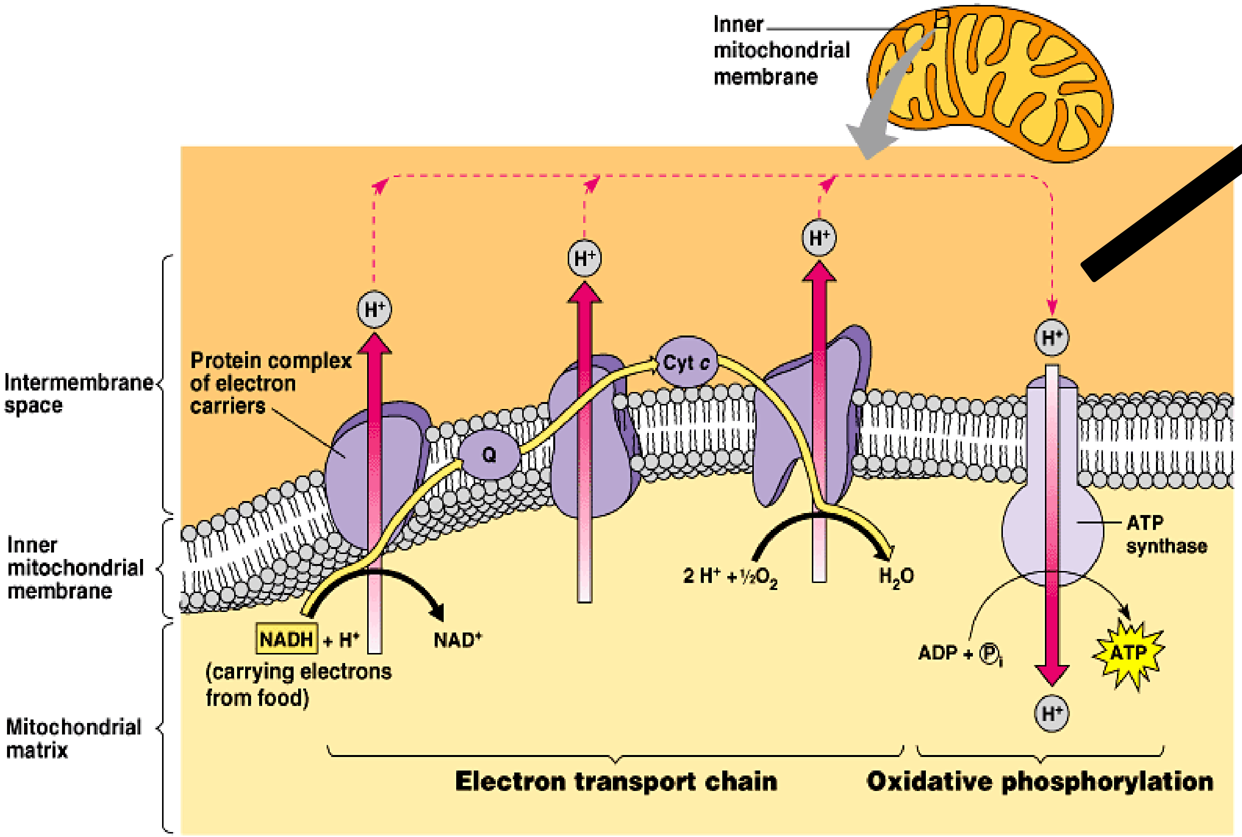
Complex V
(ATP synthase Complex)

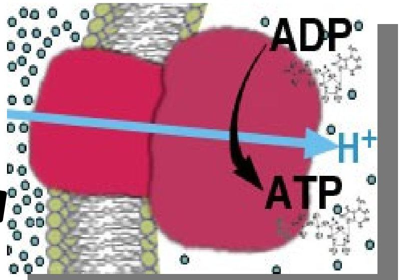


Chemiosmotic Model

How is oxidation coupled to production of ATP?

Peter Mitchell Proposed chemiosmotic



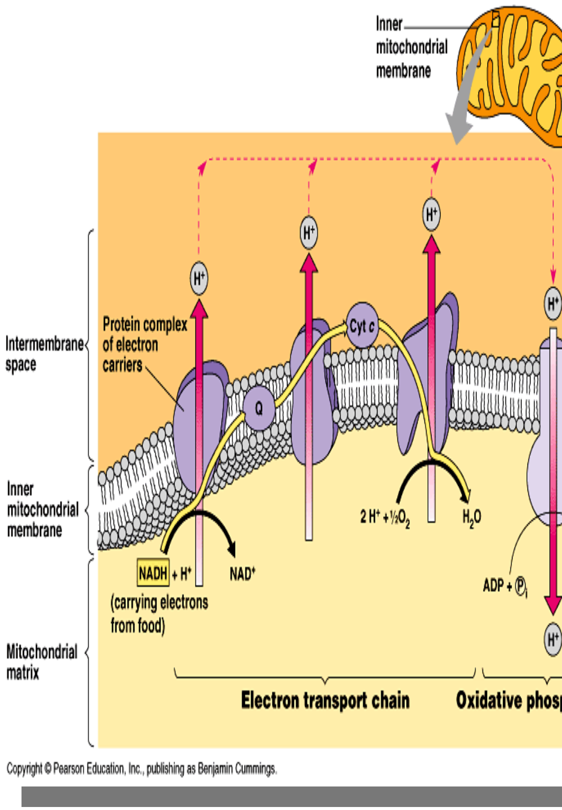


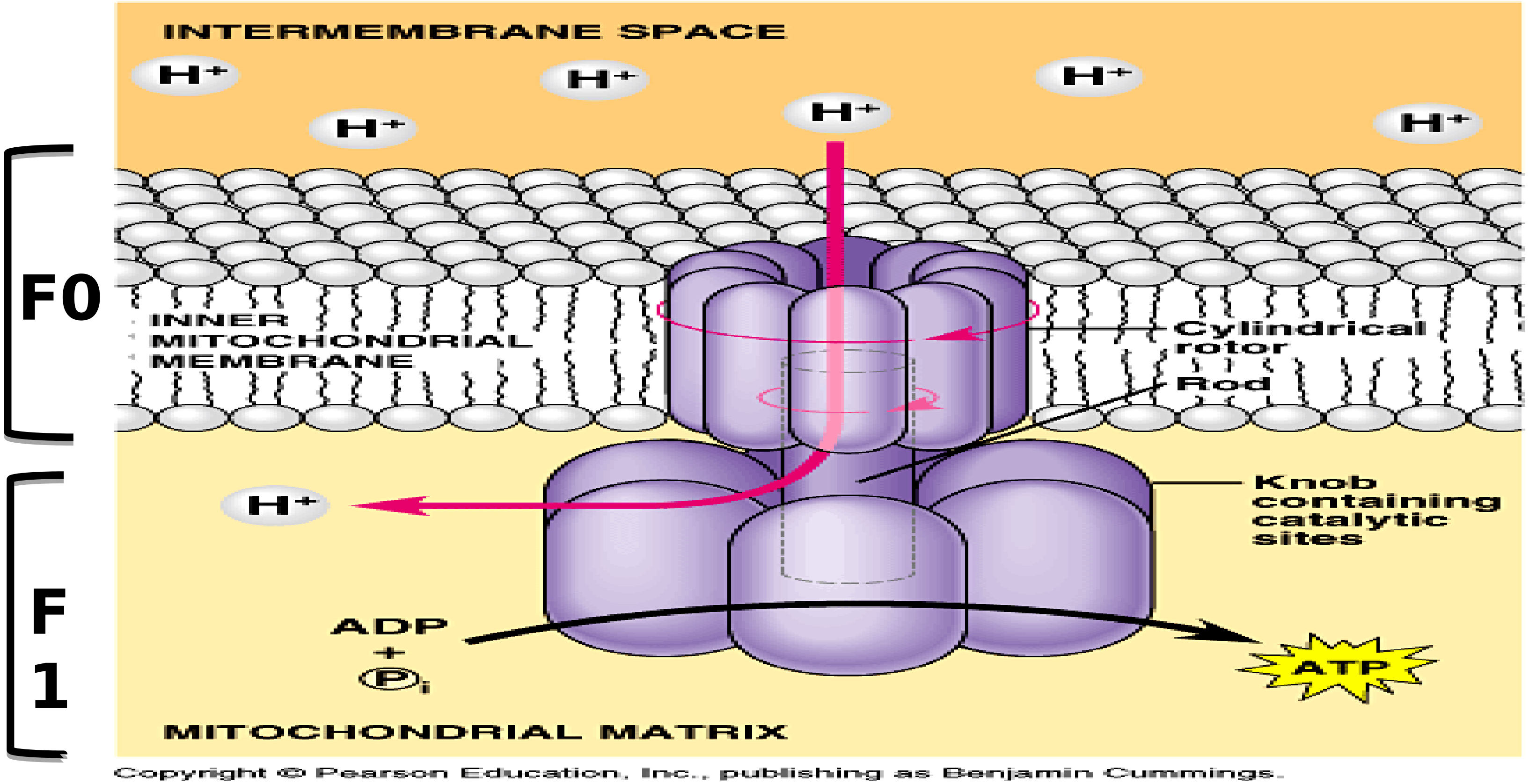
1920-1992

Chemiosmotic Model

The theory postulates that the **energy obtained from oxidation** is coupled to the **translocation of protons H+** from the mitochondrial matrix to the inter-Membrane space.

- **Complexes I, III, IV act as proton pumps.**
- So an **electrochemical gradient** is created across the inner mitochondrial membrane. **(outside is +ve, inside is negative)**





Scattered on the surface of the inner mitochondrial membrane are units of the ATP-synthase.

It is inhibited by oligomycin.

P/O ratio

It is a measure of the **number of ATP produced** during transfer of

2 e^- in the ETC.

For 2 electrons transferred through the ETC there are **protons** pumped out side at 3 *complexes (**I, III & IV**)* if it is through **NADH**

&The **protons are** pumped outside at **only** *2 complexes (**III & IV**)* if it is through **$FADH_2$**

When substrates are oxidized via ***NADH-linked dehydrogenases*** **3 ATP** are produced per ½ **O_2** consumed and this is given the term that they have a **P/O ratio of 3**.

On the other hand when a substrate is oxidized via a ***flavoprotein-linked dehydrogenase*** only **2 ATP** are produced and it has a **P/O ratio of 2.**

Respiratory control

1. **Mainly by availability of ADP.**

2. **Availability of electrons**:

↑ NADH/NAD ratio o ↑ $FADH_2$/FAD.

3. **Availability of O_2.**

Inhibitors and Uncouplers of Oxidative phosphorylation:

A number of compounds can specifically inhibit electron transfer at different points of the different complexes. These inhibitors will block the oxidation process and prevent the building of a proton gradient.

Complex I:

Rotenone, an insecticide binds to complex I and prevents the reduction of ubiquinone.

Amytal and other barbiturates: prevent electron transfer from iron sulfur centers to ubiquinone.

Complex II:

Malonate which acts as a competetive inhibitor of succinate.

Complex III:

Antibiotics inhibit electron transfer through complex III by Blocking transfer of electrons from Co Q to iron – sulfur proteins.

Complex IV:

Cyanide and carbon monoxide inhibit Complex IV resulting in impairment of the energy generating function of oxidative phosphorylation. Tissue asphyxia especially of central nervous system occurs leading to death.

Uncouplers of Oxidative phosphorylation

Uncouplers

- short – circuiting the flow of protons through the ATP synthase
- dissociate oxidation in the respiratory chain from phosphorylation
- oxidation rates ↑ , phosphorylation rate ↓
- result in the production of extra heat

“ fever ”

Uncoupling agents

Diverse group of compounds that are

- *Lipid soluble*
- *Act as proton carriers*

e.g. ***2,4 dinitrophenol, dinitrocresol***

uncouplers

(1) 2,4 dinitrophenol (DNP).

(2) High levels of bilirubin as in cases of hyperbilirubinemia.

(3) High level of thyroxine as in thyrotoxicosis.

(4) Snake venoms “their phospholipases”.

(5) Thermogenine

Is a Physiological Uncoupler: it is present in the inner mitochondrial membrane and provides a path for protons to return to the matrix without passing through the F0 F1 complex.

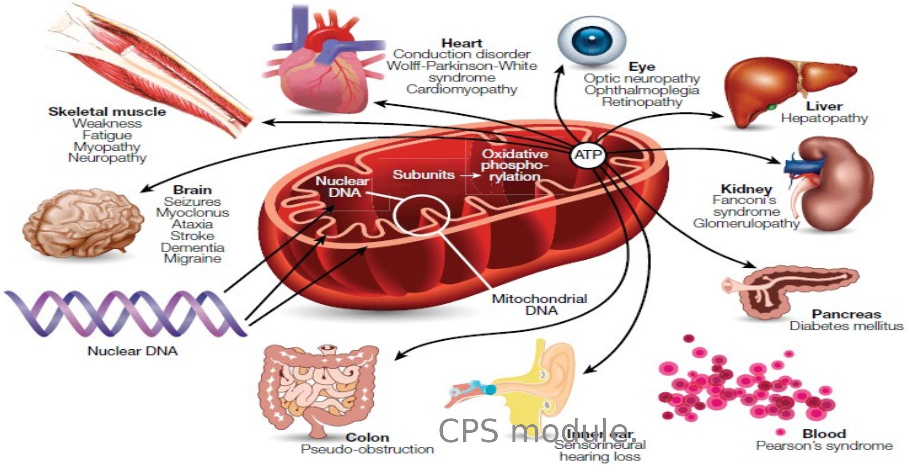
- In specific type of adipose tissues called "brown fat" In hibernating animals and in most mammals including human newborns

Mitochondrial diseases

Are heterogeneous multisystem disorders caused by dysfunctional mitochondria where *mitochondria fail to produce enough energy (ATP) for cell or organ function*

Any age

Both sex



Any Organ

Mutations in mitochondrial DNA can lead to a group of human diseases

- Inborn errors of mitochondrial DNA is inherited only from mothers.

They are a group of diseases due to deficiencies in mitochondrial transport function as carnitine system and/or in components of the mitochondrial electron transport chain

Some famous mitochondrial syndromes caused by inherited mtDNA mutations

Key features	Syndrome
Myopathy, encephalopathy, lactic acidosis and stroke-like symptoms	**MELAS**
and Retinitis pigmentosa Neuropathy, Ataxia, Ptosis	**NARP**
Myoclonic epilepsy with red ragged fibers on muscle biobsy, hearing loss and lactic acidosis	**MERFF**

Some famous mitochondrial syndromes caused by inherited mtDNA mutations

Key features	Syndrome
deafness Diabetes mellitus and	**MIDD**
A rapid decline in function marked by seizures, altered state of consciousness, dementia and ventilatory failure	$ **Leigh**

- ***Due to clinical variability existing with mtDNA mutations***
- **Many patients do not fit into one of the famous syndromes**

Mutations in mitochondrial DNA can lead to Mitochondrial myopathies

- They are a group of diseases due to deficiencies in **mitochondrial electron transport chain** as NADH dehydrogenase, Cytochrome b or the mitochondrial F0F1 ATP synthase. It usually occurs in mitochondria

Diagnosing patients with mitochondrial disease is challenging due to

***The varied clinical presentation** **(takes many different forms)**

***Symptoms of mitochondrial disease** **(are non specific)**

First of all Mitochondrial disease is generally suspected based on one of the following

Presence of a combination of symptoms and signs involving 3 or more different body organs that are difficult to *common))* explain

C/p of a patient may Suggest a well recognized mitochondrial syndrome NOT always seen

Family history of a patient may reveal maternal inheritance pattern